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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

LF infected persons who remain at risk of developing lifelong morbidity including hydrocele, elephantiasis and lymphedema. Of the 40 million people already affected, about 15 million suffer from lymphedema. As more resources are put towards the second pillar, the interventions used need to be shown to be effective and well evaluated. In this review the effectiveness of interventions for LF related lymphedema are critically appraised and a comparison is made of evidence based prevention and treatment practices in developed countries with the goal of identifying successful practices that are transferrable across economic and cultural borders. Searches of Medline and Scopus databases were conducted in March 2013 using keywords: lymphatic filariasis, elephantiasis, lymphedema, treatment, prevention, management, home care, self care and hygiene. Papers were excluded if they were reviews or reported only on surgical interventions. 309 papers were returned and 25 papers reporting specific outcomes of interventions aimed at reducing limb volume or acute dermatolymphangioadenitis episodes were included. The reference lists of WHO publications, found reviews and other excluded papers were searched for reports of original interventions and a further 9 papers were included. RCT's, interrupted time series studies and case series reports were most commonly used to evaluate drug interventions, education in self care including frequent washing and drying, elevation and exercise, and the use of pneumatic pumps or other health worker applied treatments. Level of evidence of studies was found to be generally poor; however most studies reported improvements when basic lymphedema management was carried out, especially washing and drying of affected limbs. One study indicated that lymphedema education can improve MDA compliance, but this concept requires further investigation. This review will contribute to acceleration of the impact of the second pillar of the GPELF through drawing together knowledge in lymphedema prevention and management approaches in both LF endemic and developed countries.

253

OPHTHALMOLOGICAL FINDINGS FROM A STUDY COMPARING THE EFFECT OF A SINGLE DOSE OF 150 MG/KG IVERMECTIN AND OF 8 MG MOXIDECTIN ON MICROFILARIA LEVELS IN *ONCHOCERCA VOLVULUS* INFECTIONS

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Control of onchocerciasis as a public health problem in Africa relies on annual ivermectin (IVM) mass treatment. Recently, elimination of transmission was added to control programme objectives. Moxidectin (moxi), a veterinary anthelmintic, is in development to assess whether it is safe for mass treatment with an effect on *Onchocerca volvulus* macrofilaria sufficiently higher than IVM's to reduce treatment rounds to elimination and/or to result in elimination where IVM cannot. In the randomized, double-blinded Phase 3 study in areas in Liberia, Ghana and the DRC without IVM mass treatment, a single dose of 8 mg moxi or IVM was given to 978 and 494, respectively, males and females ≥12 years with ≥10 microfilaria/mg skin (mf/mg). Baseline ophthalmological manifestations included symptoms of *O. volvulus* infection (incl. dead microfilaria in the cornea, punctate opacities, cotton wool spots, eye pruritis) as well as glaucoma, cataract, ocular infections, and other problems. More than 10 microfilaria in the anterior chamber (mfAC) were present pre-treatment in 130 (13%) of moxi treated and 75 (15%) of IVM treated. The number of mfAC and live microfilaria in the cornea (mfCL) (mean±SD) in these subjects were, respectively, 26.4±20.0 and 10.2±8.2 among moxi treated and 26.6±18.4 and 9.1±8.5 among IVM treated. MfAC levels (mean±SD) were 20.4±19.4, 4.7±10.9, 0.5±2.4, 0.3±2.2 and 0.1±0.6 at 4 days, 1, 6, 12 and 18 months after moxi treatment and 25.8±22.8, 8.6±17.7, 0.9±5.9, 1.2±6.5 and 1.7±10.1 after IVM treatment, respectively. MfCL levels (mean±SD) were 7.5±6.3, 0.6±2.3, 0.0±0.12, 0.0±0.0 and 0.0±0.2 at 4 days, 1, 6, 12 and 18 months after moxi treatment and 7.0±7.2, 1.2±4.0, 0.2±1.3, 0.0±0.1 and 0.0±0.0 after IVM treatment, respectively. While the skin mf levels and proportion of subjects with detectable skin mf at 1, 6, 12 and 18 months after moxidectin were significantly lower ($p<0.0001$) than after ivermectin treatment, the differences in the change in mfAC between the treatment groups was not significant ($p=0.12$ for month 12).

254

DIFFERENCES IN EOSINOPHIL-RELATED PATHOGENESIS UNDERLIE THE VARIED CLINICAL PRESENTATIONS OF *LOA LOA* INFECTION BETWEEN TEMPORARY RESIDENTS AND THOSE INDIGENOUS TO *LOA*-ENDEMIC AREAS

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Previous studies have suggested that *Loa loa* infections in inhabitants of *Loa*-endemic areas (END) have marked differences in clinical presentation and post-treatment reactions compared to those of temporary residents (TR). Many of these differences are thought to be immune-mediated. To define the underlying pathophysiology of these differences in clinical presentation, we conducted a retrospective analysis of 186 patients with loiasis seen at the National Institutes of Health. Among the 186, 42 were raised in *L. loa*-endemic regions while 144 were visitors to these same regions. The initial clinical presentation differed markedly between the two groups with only 50% of END having a history of Calabar swelling compared to 82 % of TR ($p<.001$). In contrast, the END were much more likely to have had eyeworm (71% compared to 15%, $p<.001$) and were more likely to have microfilaremia (74% compared to 22% in the TR, $p<.001$). The absolute eosinophil counts (AEC) were markedly different between the groups; the geometric mean (GM) AEC in TR were 1532/uL compared to 706/uL in the END ($p=.0003$). Along with the quantitative increase in AEC, individuals in the TR group showed evidence of increased eosinophil activation in that the serum levels of all four eosinophil granule proteins (EDN, EPO, ECP, and MBP) at baseline were higher in the TR group compared to END. In addition, the levels of EDN and EPO in the TR group at presentation were positively correlated with both the AEC and IgE levels ($p <.05$). Baseline serum levels of eosinophil-associated cytokines, including IL-4, Eotaxin, GM-CSF, and IL-5 were all found to be elevated in the TR group compared to the END group ($p<0.05$ for all cytokines). These data extend earlier observations related to immunologically based differences between TR and END of Loa-endemic regions. Additional data concerning eosinophil-related pathogenesis of loiasis and changes in AEC and eosinophil-related cytokines following treatment will be discussed.

255

LOA LOA INFECTION MODULATES THE T CELL MEMORY RESPONSE TO MYCOBACTERIA

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Chronic filarial infections have been associated with a type of bystander suppression felt to be mediated by IL-10-producing Th2 or adaptive

T regulatory cells. To assess the heretofore unexplored dynamics of homeostatic and antigen (Ag)-specific memory T cell compartments in the context of filarial infection, including the recently described memory T stem cell compartment --reported to persist stably long-term -- we used multiparameter flow cytometry on PBMCs from 17 microfilaremic *Loa loa* (Ll) -infected (Loa+) and 10 Ll–uninfected (Loa-) subjects following stimulation with filarial (BmA) or mycobacterial (PPD, ESAT-6, CFP10) Ag. Using intracellular CD154 to mark Ag-activated CD4+ T cells, we demonstrated that the Loa+ group compared to the Loa- group showed an increase in CD3+ CD4+CD154+IL-10+ cells (Median increase on stimulation (M)=0.1230% vs -0.14%, p=0.03) in response to BmA as well as to PPD (M=0.1237% vs. -0.2583%, p=0.009), and an increase in CD4+CD154+IL-4+cells with CFP10 (M=0.3% vs. 0%, p=0.0266). Loa+ subjects showed no expansion of CD4+CD154+CD45RO+IL-4+, IL-10+ or IL4+IL-10+ producing central memory (Tcm, CCR7+CD27+), effector memory (Tem CCR7-CD27-), transitional memory (Ttm CCR7-CD27+) or stem cell memory (Tscm, CD45RA+CCR7+CD27+CD95+) cells at homeostasis or on stimulation with either BmA or mycobacterial Ag. However, an increased frequency of a poorly studied antigen-experienced population of CD4+CD154+CD45RA+CCR7+CD27+CD95-IL-4+ producing cells were seen in Loa+ individuals in response to BmA (M=0.15% vs. 0.056 %, p=0.01) as well as to PPD (M=0.1% vs. 02%, p=0.03) and CFP10 (M=0.15% vs. 0.14%, p=0.007) compared to the Loa- subjects. These data suggest that CD154 can be used to quantify Ag activated CD4+Th2 responses to parasitic and bystander Ag. We also have been able to identify a novel Ag-experienced CD45RA+ cell population capable of producing Th2-related cytokines that may be a new CD4+ 'memory' cell capable of modulating the effector T cell response to parasite and bystander Antigens.

256

IN VITRO VALIDATION STUDY OF PEPTIDES FROM IN SILICO ADVANCED EPITOPE MAPPING PREDICTIONS TO DELINEATE T EFFECTOR EPITOPES WITHIN STAGE-SPECIFIC *BRUGIA MALAYI* SECRETED PROTEINS

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The complex host-pathogen interactions between humans and the causative agents of lymphatic filariasis--*Wuchereria bancrofti, Brugia malayi*, and *Brugia timori*--are well known to favor the survival of both host and pathogen. However, the quality of life for chronically infected humans can be severely affected by the disabling disfigurements manifesting upon death of adult filarids. Efforts continue to be made for the development of an effective vaccine to combat these pathogens. Nonetheless, it is the same host-pathogen relationship that makes the traditional approach to vaccine design, with respect to immunogenic antigen discovery, extremely difficult to apply against the multicellular filarids. Thus, an alternative approach to the discovery of immunogens was the foundation of this study. This approach consisted of screening pathogen proteins with immuno-informatics algorithms and testing predictions with immunological assays. Over 900 protein sequences believed to be secreted during the L3, adult male, adult female and/or microfilarial life stages of *B. malayi* were screened. A peptide library of 20 putatively immunogenic epitope clusters was created and tested for proof-of-concept using a 10 day re-stimulation assay on naive peripheral blood mononuclear cells (PBMCs) with different peptide concentrations ranging from 25mg/ml to 200mg/ml. In response to peptide stimuli, cytokine specific secretions were assessed by ELISpot assays. Specific responses, such as interleukin-4 and interleukin-5 secretions, varied among the different patient PBMCs samples. Optimal cytokine production also varied among different peptide concentrations where a subset of peptides yielded the strongest cytokine secretion at lower concentrations. Results demonstrate the potential of immuno-informatics software as an alternative, low cost approach to discovering filarid immunogens as well as the feasibility of testing predictions with immunological assays to further characterize the specific effector T cell responses by cytokine markers indicative of CD4+ T cell recognition.

257

H1 RECEPTOR BLOCKADE ENHANCES EOSINOPHIL-MEDIATED CLEARANCE OF FILARIAL NEMATODES

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Recently, we demonstrated that chronic administration of fexofenadine, an H1-receptor blocker, reduces adult worm burdens in BALB/c mice infected with Litomosoides sigmodontis, a tissue-invasive filarial infection of rodents that lives for months in immunocompetent BALB/c mice. In this study, we sought to determine the mechanism by which this occurs. In vitro addition of histamine or fexofenadine to L3 stage worms did not alter worm motility or duration of survival in culture, suggesting that fexofenadine does not directly affect the viability of L3 worms. To evaluate the *in vivo* effects of fexofenadine, we infected mice by subcutaneous injection of 40 L3 stage worms and treated BALB/c mice with an average of 20 mg/kg per day of fexofenadine administered in drinking water. Consistent with our previous studies, worm counts dropped significantly in fexofenadine treated animals (mean number adult worms = 18 in untreated mice vs 5 in fexofenadine treated animals, p = 0.001). Next, we conducted multicolor flow cytometry to quantify eosinophil numbers in the pleural space, the site of adult worms. In contrast to untreated mice, in which mean eosinophil count in the pleural space was 0.6 x 106, fexofenadine-treated mice had 2.4 x 106 pleural space eosinophils. To determine if eosinophils were important for fexofenadine-treated mice, we next evaluated whether fexofenadine decreases worm burden in eosinophil-deficient dblGATA mice. Eosinophil deficient mice exhibited similar numbers of adult worms 8 weeks post-infection as wild-type mice (mean 14 adult worms per mouse in both groups). In contrast to studies in wild type mice, fexofenadine treatment did not alter adult worm burden in dblGATA mice (mean 17 adult worms per mouse, p compared to untreated dblGATA mice = 0.4). These results suggest that fexofenadine enhances clearance of adult filarial worms by augmentation of the eosinophil response.

258

EVIDENCE FOR INVOLVEMENT OF CYS-LOOP LIGAND-GATED ION CHANNEL GENES IN REPRODUCTION OF FILARIAL WORMS

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Several commonly used anthelmintic drugs (macrocyclic lactones or MLs, and nicotinic agonists) target members of the CLGIC gene family. MLs (e.g., ivermectin (IVM) which binds to glutamate chloride-gated channels, GluCls) and nicotinic agonists (e.g., levamisole which binds to nicotinic acetycholine receptors, nAChR), have their greatest activity against microfilariae (Mf) of filarial nematodes such as *Onchocerca volvulus*. These drugs also affect fecundity in some species. This suggests that GluCl and nAChR may have a role in reproduction, but the nature of that role is poorly understood. Our prior studies have shown that gene expression patterns in filarial nematodes often correlate with biological function. We used *in situ* hybridization to study gene expression patterns for seven CLGIC genes including IVM-sensitive subunits from GluCls and levamisole-sensitive receptor from nAChR in the filarial parasite *B. malayi*. Six of these genes were strongly expressed in early developing embryos (morula and pretzel stage) in the uterus and spermatogonia in testis, weakly expressed in later stage embryos, and partially or not expressed in stretched microfilariae or mature sperm. Most of these genes were strongly expressed in the wall of the uterus and vas deferens adjacent to stretched Mf or mature spermatozoa. Some of these genes had unique expression patterns. For example, AVR-14B, which encodes an IVM-sensitive GluCl channel subunit, was only expressed in female worms, and Bm1_40515,

a member of cAChR gene family was only expressed in male worms; Bm1_35890, a *C. elegans* orthologue of *unc-29* encoding the levamisole-sensitive receptor was strongly expressed in both male and female worms. In summary, *in situ* localization results are consistent with the observed suppressive effects of IVM and levamisole on embryogenesis in *B. malayi* and provide the molecular evidence for involvement of CLGLC genes in filarial reproduction. Our studies have also provided new insights regarding the sites of action of MLs that are broadly active against nematodes and arthropods.

259

WHOLE GENOME SEQUENCING OF *WUCHERERIA BANCROFTI* FROM AN INFECTED HUMAN BLOOD SAMPLE

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Lymphatic filariasis (LF) is a major threat to human health in the tropics, leading to the disfiguring and debilitating conditions hydroceles and elephantiasis. There are approximately 1.2 billion people living in LF endemic countries where 120 million people currently suffer from the disease. The parasites that cause LF are separated into two genera, *Brugia* and *Wuchereria*, with *W. bancrofti* (Wb) responsible for ~90% of LF cases. *Wb* has been neglected in genomic studies due to complications in sample collection and preparation, such as lack of adult stage samples. Therefore, in order to obtain sufficient material to perform genome sequencing Wb larval stages (microfilaria) must be sampled from infected host blood. These samples will inevitably contain human leukocytes and therefore human DNA contamination. Here we present a method to reduce human DNA contamination and subsequently produce high quality genome sequence for Wb directly from an infected human blood sample. Twenty-five patients were selected to have varying concentrations of mircofilaria in the blood. For each patient we used a set number (1, 2, or 3) of Percoll density gradient centrifugation steps to remove cells containing human DNA. After DNA extraction we quantified the concentration of human to Wb DNA by way of qPCR. We determined that only a single Percoll treatment was needed to optimize reduction of human DNA while still maximizing the recovery of Wb DNA. Successive treatments decreased the yield of Wb DNA while having minimum effect on human DNA concentration. We selected a single treated sample for whole genome sequencing based on the results of qPCR. Whole genome sequenced was performed on the Illumina HiSeq2000 platform that generated 200-million paired-end reads of 150 base pairs each. The resulting sequences were mapped to human reference Hg19 and the previously assembled contigs of Wb from Mali. Human DNA comprised only 40% of our sequence reads with remaining reads belonging to a combination of Wb (58%) and its bacterial symbiont, *Wolbachia* (2%). Removal of human DNA resulted in an average nucleotide coverage of 200x across the Wb genome, which is 30-50 times higher than that currently available for the Wb genome from Mali. This sequence of the Wb genome is unique in being derived from an individual infected whole blood sample and is the first Wb genome available from Papua New Guinea.

260

DISCOVERING NOVEL PARASITICIDES FROM FILAMENTOUS FUNGI

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Mycosynthetix owns one of the largest libraries (55,000 strains) of filamentous fungi in the world. These organisms were isolated from substrates sourced from a wide variety of ecosystems and include many strains from tropical rain forests where there is a high degree of biodiversity. Our primary purpose is to exploit the medicinal and pharmaceutical potential of this large library. The targets of the screening efforts include cancer, bacteria, fungi and several human and animal parasites. The latter include malaria, trypanosomes, leishmania, soil transmitted helminths (STHs) and filarial worms which infect humans and animals. We have established validated whole organism assays to determine activity of both pure compounds and semi-purified extracts of fungi against GI tract nematodes, filarial worms, adult arthropods and mosquito larvae. Pure compounds have been shown to have high activity against one or more of the following; *Haemonchus contortus*, *Cooperia onchophora*, *Strongyloides stercoralis, Brugia pahangi* (Bp) and *Dirofilaria immitis* (Di) microfilaria (Mf) and L3 and L4 stages and *Aedes aegypti* larvae. There is little crossover of activity between the endo- and ectoparasites. Several compounds not only had high potency against BpMf but also caused high mortality within 24 hours. A small library of carefully selected fungal metabolites was tested in the BpMf assay at 50 ppm giving an overall hit rate (ED_{100}) of 96.1% at 120 hrs. Upon down titration, 30/58 compounds were fully active at 3.125 ppm (3-6 μM) or less and a number had nanomolar activity. Several of these 30 were also active against DiMf. One compound showed potent activity against Bp L3 and L4 and DiL3, and several others are being tested. Results from several mammalian cell lines coupled with associated anti-parasitic data have identified a number of compounds with high potency and a promising *in vitro* therapeutic index.

261

COEVOLUTION AND PARTNER FIDELITY IN PARASITE - BACTERIAL SYMBIOSES

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Parasite - bacterial interactions are common in nature, and in particular, beneficial symbiotic interactions between these organisms can have large impacts on parasitic diseases and parasite biology, ecology and evolution. Therefore it is essential that we understand the fundamental pressures that influence the formation and maintenance of parasite - bacterial symbioses to fully comprehend how these interactions contribute to the evolution of clinically relevant parasites (e.g. filarial nematodes). Toward this, we utilized the *Steinernema* nematode - *Xenorhabdus* bacteria model system to study the influence of bacteria on parasitic success and the evolution of parasite -bacterial associations. We found co-cladegenesis (congruence) between the phylogenies of *S. feltiae* nematode isolates and their natural *X. bovienii* bacterial symbionts, which are considered strains of the same species by current metrics. Robust co-cladegenesis indicates that there is strong selection for maintenance of the symbiosis and that coevolution is likely occurring. To test this possibility we experimentally measured the impact of the bacterial strains on the relative success of parasitic infection and were able to detect differences between the nematode - bacterial combinations, indicating that bacterial strains may differ in their contributions to parasitic infection success. In addition, the differences reflected a pattern that is consistent with coadaptation occurring at a sub-species level. The better success of parasites associated with native partners, relative to non-native partners, could serve to reinforce partner fidelity between the pair, and this in turn can influence the larger phylogenetic patterns that we observe. This suggests that interactions between bacterial strains and parasites may impact the parasite evolution. In addition, our data highlight that interactions between parasite and bacterial populations likely differ and should be considered in our studies of the impacts of bacteria on parasite biology and parasitic diseases.

262

RAPID SCALE UP WITH HIGH TREATMENT COVERAGE FOR LYMPHATIC FILARIASIS ELIMINATION IN MALAWI: IMPACT ON INFECTION RATES AFTER 3 TO 4 TREATMENT ROUNDS

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Lymphatic filariasis (LF) caused by *Wuchereria bancrofti* is highly endemic and widespread in Malawi where 26 of the 28 districts are endemic. The national LF elimination programme was launched in 2008 to protect 14 million people at risk of acquiring the infection. During 2008, 2.7 million people were treated in eight of the 26 endemic districts with a reported drug coverage of 80.5%. In the following year, MDA scaled up dramatically to achieve 100% geographic coverage and 10.8 million people were treated. Treatment coverage was 80% or higher for the subsequent treatment rounds carried out in all endemic districts. To test the hypothesis that rapid scale-up and high treatment coverage accelerates the transmission interruption process we conducted sentinel site surveys to monitor impact on infection rates. Mapping surveys to identify implementation units for MDA were conducted in 2000 and 2003 with ICT cards and the results informed the identification of 15 high risk villages which serve as sentinel sites for impact assessment. The overall microfilaremia (MF) baseline rates from 4738 people tested was 1.6%, ranging from 0.5% in Zomba in the Southern Region, to 9.1% in Karonga in the Northern Region. The Overall MF rate decreased by 87.5% after 3-4 rounds of MDA and MF was not detectable in 10 of the 15 sentinel sites where 5263 people were tested in 2012. Only one of the sentinel sites had MF rate above 1%. All endemic districts in Malawi will conduct their 5th or 6th annual MDA in 2013. If the high treatment coverage is maintained and MF prevalence continues to decrease, all sentinel sites will qualify for transmission assessment surveys to determine whether to stop mass treatment.

263

CRITERIA FOR TESTING DEVELOPMENT OF RAPID DIAGNOSTIC TEST TO SUPPORT ONCHOCERCIASIS CONTROL AND ELIMINATION PROGRAMS

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There is often significant discrepancy between analytical performance of diagnostic tests determined under laboratory conditions and operational performance of the same tests. Failures induced by real-world circumstances may compromise test performance and subsequent availability to the communities in need. In order to address potential failures from the research and development phase through final product release, we have developed data-supported criteria that are relevant to successful test deployment into a field setting. We have applied these criteria to the development of a rapid test for exposure to the parasitic worms *Onchocerca volvulus* (Ov) which causes onchocerciasis, or river blindness. A major cause of preventable blindness around the world, Ov is transmitted to humans through the bite of the blackfly and typically affects poor, rural communities near fast-flowing streams and rivers. Mass administration campaigns of the drug ivermectin have significantly reduced disease burden in some regions to the extent that elimination has become possible. To enhance surveillance of onchocerciasis exposure and infection, we have developed rapid diagnostic test (RDT) prototypes of a clinically-validated assay detecting IgG4 antibodies specific to a previously validated Ov antigen, Ov16. These RDTs have been advanced to readiness for field studies through evaluation with a collection of development criteria designed to anticipate common user failures, operational testing challenges, and options in sample collection. We describe the development of a positive control human anti-Ov16 monoclonal IgG4 for use by the onchocerciasis community and our performance and stability data for application to quality control and assurance of Ov16 RDTs. We present data informing the development criteria, usability feedback, development of instructional material, and performance of the test through induced failure modes and rigorous storage and operational conditions. All of these measures ensure that a robust and user-friendly Ov16 test is developed and brought to the field.

264

IMPACT OF REPEATED IVERMECTIN TREATMENTS AGAINST ONCHOCERCIASIS ON THE TRANSMISSION OF LOIASIS: AN ENTOMOLOGICAL EVALUATION IN CENTRAL CAMEROON

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Mass treatments with ivermectin (IVM) against onchocerciasis started in Cameroon in the early 1990s. In the Lekie division, distributions with low coverages started in 1993, but it was not until 1999 that the whole area was covered using the community-directed treatment with IVM (CDTI) strategy. In this area, onchocerciasis is coendemic with loiasis (*Loa loa* filariasis) and, since 1995, cases of serious adverse events (SAEs) were recorded in patients presenting a high *Loa* microfilaremia (>30,000 microfilariae per mL blood). An entomologic study on loiasis was conducted from May 1999 to April 2000 in one village of the area (Kokodo). Chrysops were caught during 3 consecutive days every week to assess the proportions of flies harboring *Loa* larvae of any stage (infection rate), stage 3 larvae (L3) in the head (infective rate, IR), and L3 whatever the location (potential infective rates, PIR). The mean numbers of L3 per infective and per potentially infective fly (MHL3 and MFL3) were also assessed. A second entomologic study was carried from March to June 2012 to evaluate the impact of 13 years of CDTI on the transmission of loiasis. The sites and methods were identical to those used in 1999-2000. The indices measured during this 4-month period were compared with those obtained during the same months in 1999-2000. The biting density was almost three-fold higher in 2012 than in 1999-2000. There was a significant reduction in the infection rates between the two periods (from 7.1 to 3.0%, p=0.001). The differences in the IRs, PIRs, MHL3 and MFL3 were not significantly different between the two periods. The infection rate remains high in spite of its significant reduction, and the stability observed in the other indices after 13 years of CDTI shows that transmission of L. loa is still active in the area. A parasitologic survey should be conducted to evaluate whether these entomologic results correspond to high levels of infection in the population.

265

DISCOVERY PROTEOMICS: UNRAVELLING THE MUTUALISTIC SYMBIOSIS OF *WOLBACHIA* AND THE FILARIAL NEMATODE *BRUGIA MALAYI*

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The parasitic nematode *Brugia malayi* is a causative agent of lymphatic filariasis, a disfiguring disease affecting over 120 million people worldwide. *B. malayi* exists in a mutualistic symbiotic relationship with the α-proteobacterium *Wolbachia*. We have applied a global proteomic profiling approach to investigate the molecular basis of this symbiosis. Adult female *B. malayi* in the mammalian host *Meriones unguiculatus* were sampled at multiple time-points post-antibiotic depletion. Deep proteome mining combined with high-resolution mass spectrometry was used for comprehensive proteome profiling of *Wolbachia*/worm at these selected time-points. *Wolbachia*/worm ratios were also monitored by qPCR. In-solution tryptic proteolysis coupled with reversed phase liquid chromatography and analysis by high-resolution mass spectrometry provides a powerful tool for global proteome profiling. This initial shot-gun approach has been optimised to include an extensive peptide pre-fractionation step using the Agilent 3100 OFFGEL fractionator system. Using a combination of pre-fractionation and established proteomic workflows we observed improved proteome coverage by an increase in peptide/protein identification. Following basic analysis through established bioinformatics pipelines, transcriptomic, proteomic, and published datasets will be integrated in a systems biology approach with the objective of understanding the molecular basis of the mutualistic *Wolbachia*/*B. malayi* symbiosis.

266

A•WOL MACROFILARICIDAL DRUG DISCOVERY - LEAD OPTIMIZATION OF HIT CHEMOTYPES WITH ANTI-*WOLBACHIA* EFFICACY IDENTIFIED DURING THE SCREENING CAMPAIGN OF FOCUSED ANTI-INFECTIVE AND DIVERSITY-BASED LIBRARIES

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There is an urgent need to develop a novel treatment for filariasis, and targeting *Wolbachia* provides safe macrofilaricidal activity with superior therapeutic outcomes compared to standard anti-filarial treatments. In order to turn this into a public health tool suitable for control programs, the A•WOL Consortium utilises *in vitro* cell and nematode assays, followed by *in vivo* assays, to screen chemical libraries for anti-*Wolbachia* activity. During the screening campaign, the *Wolbachia in vitro* assay has been developed using Operetta high content imaging with automation, in order to radically increase throughput. Using this approach, screening of 10130 compounds, from libraries of focused anti-infectives and registered drugs, has identified 632 hits, with 324 showing improved efficacy over doxycycline. The diversity-based approach has involved procurement and ongoing screening of >510k compounds from large libraries. Progression along the pipeline selects for hits which also show activity against nematode *Wolbachia in vitro* and *in vivo*. Hits are then scrutinised to determine suitability for structure-activity relationship assessment, and narrow-spectrum anti-*Wolbachia* activity in order to select the best candidates to take forward. These extensive screening activities have generated four independent lead series chemotypes based on equivalent or improved activity over doxycycline in *in vitro* and *in vivo* models (as absolute potency or duration of treatment to deliver *Wolbachia* elimination), chemical tractability and prior experience with the class. These lead series are progressing through a rigorous lead optimisation and candidate selection process, using iterative cycles of medicinal chemistry and biological testing in order to deliver at least one novel pre-clinical candidate and a chemically distinct back-up, aligned with our Target Product Profiles for an anti-*Wolbachia* macrofilaricide. Ongoing screening activities aim to provide additional, chemically diverse hits, with one-order improvement in absolute potency or significant shortening of treatment (≥25%).

267

DEVELOPMENT OF A FILARIAL MURINE INFECTION MODEL TO SCREEN ANTI-*WOLBACHIA* DRUGS

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Filariasis is a priority neglected tropical disease affecting >150 million of the World's poorest populations. New drugs require testing *in vivo* if control of filariasis is to be sustained. It is possible to passage filariae of the genus Brugia in gerbils including the human pathogen, *Brugia malayi*. However inbred lab mice are refractory to full development of B. malayi. It would be desirable to establish a robust murine model of Brugian filariasis to screen new pharmacological agents, including anti-microbials that target the nematode endosymbiont, *Wolbachia*. The advantages of this include: reduced costs of manufacturing sufficient drug for testing, lower animal husbandry costs and use of a rodent with a well-characterised pharmacokinetic profile. Previously it has been reported that Brugia spp consistently develop through to fecund adult infections in the peritoneum of lymphopenic mice, such as Severe Combined Immuno-Deficiency (SCID) mice. Our own experimentation has identified that modulating eosinophil function greatly enhances survival of larvae. Therefore we tested male mice with impaired eosinophil responses (interleukin-4 receptor alpha; IL-4Rα-/-, IL-4Rα-/-IL-5-/-) and SCID mice, all on a BALB/c background, for their permissiveness to fecund Brugia infection. Whilst all strains were permissive at +5 weeks (juvenile adult stage) typically yielding between 20-40% recoveries of initial inoculate, adult infections of B. malayi waned past this stage in IL-4Rα-/- , so much so that IL-4Rα-/- inconsistently retained permissiveness to fecund infections. Contrastingly both IL-4Rα-/-IL-5-/- and SCID maintained 10%-20% recoveries of microfilariae (mf) producing Brugia adults to beyond +20 weeks. Therefore IL-4Rα-/-IL-5-/- and SCID were validated for anti-*Wolbachia* drug screening using oral administration of tetracyclines. *Wolbachia* expansion during L3 to L4 development could be successfully blocked in both strains by oral daily tetracycline administration. Also both strains could be successfully used to deplete *Wolbachia* by >1log (a level of permanent sterilisation) and prevent mf release in adult female Brugia after 4-6 weeks oral administration of tetracycline. Thus we have demonstrated that selective deficiency of adaptive immune responses controlling eosinophil recruitment or SCID can yield a highly permissive mouse model of filariasis validated for anti-*Wolbachia* pre-clinical screening.

268

INTESTINAL HELMINTHIASIS DIAGNOSED IN DAKAR, SENEGAL

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Parasitic infections, caused by intestinal helminths and protozoan parasites, are among the most prevalent infections in humans in developing countries. In developed countries, protozoan parasites more commonly cause gastrointestinal infections compared to helminths. Intestinal parasites cause a significant morbidity and mortality in endemic countries. Helminths are worms with many cells. Nematodes (roundworms), cestodes (tapeworms), and trematodes (flatworms) are among the most common helminths that inhabit the human gut. The goal of this study was to

determine the prevalence of digestive helminthiasis among patients referred to the laboratory of parasitology and mycology at Le Dantec hospital in Dakar for examination of stool samples from 2004 to 2009. Out of 1 526 direct stool examinations (Ritchie and Baerman techniques) analyzed at the laboratory of parasitology and mycology of Le Dantec hospital from 2004 to 2009, 310 were positive for intestinal helminthiasis (20.3%). The main species found were: *Ascaris lombricoïdes, Trichuris trichiura, Strongyloides stercoralis, Taenia saginata* and *T. solium*. Most of the patients had a single parasite (90.1%, versus 9% with two and 0.9% with three). Men are infected more often than women, accounting respectively for 58% and 42% of the infections, for a sex ratio of 1.38. Children aged 10 to 15 years had the highest prevalence of infection: 34.5%. The results show that digestive helminthiasis is endemic in Dakar, where it is necessary to implement: deworming campaigns, health education and environmental improvement.

269

PREVALENCE OF SOIL TRANSMITTED HELMINTHS AFTER MASS ALBENDAZOLE ADMINISTRATION IN AN INDIGENOUS COMMUNITY OF THE MANU JUNGLE IN PERU

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Few data are available on soil transmitted helminths in indigenous populations in the Amazon. Albendazole mass administration has not been carefully evaluated in that context. We report the prevalence of soil transmitted helminths, anemia, and malnutrition in a Matsigenka ethnic group from Yomibato in the Peruvian Amazon rain forest. All participants received 2 doses of albendazole on consecutive days, 3 months and again 2 weeks, before data collection. In total, 290 subjects from 52 families were studied. Most were female (53%), 22% were under 5 years old, 39% between 5 and 19 years, 22% between 20 and 35 years, and 17% were older than 35 years. *Trichiuris* (30%), hookworm (19%), *Ascaris* (18%), and *Strongyloides* (6%) were the most common helminths. *Enterobius* (4%), *Fasciola* like eggs (2%), *Capillaria hepatica* (2%), and *Hymenolepsis nana* (1%) were also found. Mean (±SD) egg counts/gram of stools were 1,194 (±1,821) for *Ascaris*, 100 (± 132) for *Trichiuris*, 31 (± 17) for hookworm. *Giardia* (28%) and *B. hominis* (46%) were common. Most (63%) participants had at least one parasite other than *B. hominis* and 50% had helminths. Subjects 5 to 19 years (53%) and 20 to 35 years (67%) old had helminths more often than those under 5 years (36%) and older than 35 years (41%) ($p=0.02$). One third (35%) of participants had anemia, which was more common in children under 5 years than in other participants (67% vs. 25%, $p< 0.001$). Stunting in children 0 to 20 years was common (70%), but wasting was not (3%). Children 0 to 10 years old were often underweight (29%). Overall, 72% of children were malnourished. *Ascaris* and *Trichiuris* mean egg counts were higher in malnourished children than in the rest, but differences were not significant ($p>0.05$). Despite repeated doses of albendazole, a higher than expected prevalence of soil transmitted helminths was found. Although, infection intensity was low, malnourished children tended to have higher egg counts. Anemia was common and associated with age.

270

MODULATION OF MYCOBACTERIAL ANTIGEN-SPECIFIC MONO- AND MULTI-FUNCTIONAL TH1, TH2 AND TH17 CELLS IN LATENT TUBERCULOSIS BY CO-EXISTENT HOOKWORM INFECTION

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It is estimated that tuberculosis (TB) infects one third of the world's population, resulting in two million deaths per year. In addition, helminth infections are estimated to occur in 1.5 billion people worldwide and a great majority of those infections are concentrated in developing nations where TB is endemic. A hallmark of helminth infections, both in experimental models and human infection, is the generation of profound T helper (Th) 2 and T regulatory cell responses that have the potential to suppress the predominant Th1 (IFN-γ-mediated) response needed to control *Mycobacterium tuberculosis* (Mtb) infection and enhance susceptibility to infection and/or disease. Given the overlapping distribution of hookworm infections and tuberculosis in a country like India, we wanted to determine the role of coincident hookworm infection on responses at steady state and on Mtb - specific immune responses in latent tuberculosis (LTB). We examined the cellular responses in individuals with latent TB with or without concomitant hookworm infection. By analyzing the expression of Th1, Th2 and Th17 subsets of $CD4^+$ T cells, we were able to demonstrate that the presence of coincident hookworm infection significantly diminished both spontaneously expressed and Mtb - specific mono - and dual - functional Th1 (IL-2, TNF-α and IFN-γ) and Th17 cells (IL-17A, IL-22 and IL-10). Hookworm infection, in contrast, was associated with expanded frequencies of mono - and dual - functional Th2 cells (IL-5, IL-4 and IL-13) at both steady state and upon antigen - stimulation. This differential induction of $CD4^+$ T cell subsets was abrogated upon mitogen stimulation. In addition, coincident hookworm infection was associated with increased adaptive T regulatory (aTreg) cells but not natural regulatory T cells (nTregs) in latent TB. Finally, the $CD4^+$ T cell cytokine expression pattern was also associated with alterations in the systemic levels of Th1 (IFN-gamma and TNF- α) and Th2 (IL-5 and IL-13) cytokines. Thus, coincident hookworm infection exerts a profound inhibitory effect on protective Th1 and Th17 responses in latent tuberculosis and may predispose toward the development of active tuberculosis in humans.

271

DETECTION OF *STRONGYLOIDES STERCOLARIS* IN FECAL SAMPLES USING CONVENTIONAL PARASITOLOGICAL TECHNIQUES AND REAL TIME PCR: A COMPARATIVE STUDY

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Strongyloidiasis is an extremely common cause of morbidity and mortality worldwide. In Egypt, the prevalence rate ranges from 1.5-11% with the increasing number of immunodeficient individuals, investigating the epidemiology and outcome of *Strongyloides stercoralis* infection is essential. Conventional diagnostic techniques don't efficiently detect the parasite. Therefore, the need for more efficient methods that improve diagnosis particularly in those at risk to develop the severe form of the disease is warranted. Stool samples were collected from 115 patients living in rural areas in Ismailia governorate, Egypt. All samples were subjected to agar plate culture (APC), Harada-Mori culture, Baermann concentration, formalin ethyl acetate concentration (FEAC), and real-time PCR targeting the small subunit of the rRNA gene. Among the stool samples analyzed, *S. stercoralis*: Harada-Mori detected 11 positive samples (9.6%), FEAC detected 13 (11.3%), Baermann concentration detected 16 (13.9%) and APC detected 18 (15.7%) samples. Real-time PCR assay detected *S. stercoralis* DNA in 23 (20%) samples. One sample was positive by APC but negative by the other parasitological methods. This sample was confirmed positive by real time PCR. Real-time PCR is a very sensitive and specific method, offering nearly a two-fold increase in the detection rate of *S. stercoralis* by FEAC. It doesn't require much time to perform (45 min for extraction and about one hour in amplification and detection), reduces the risk of infection, has the ability to detect dead larvae and easy to perform and interpret the data, but it still the most expensive method. On the other hand, real-time PCR technology is becoming available in an increasing number of centrally located research centers within low to middle-income countries. Moreover, PCR has the ability of detecting multiple pathogens simultaneously in one test using multiplex real-time PCR, thus saving time and money.

272

TRICHINELLA SERINE PROTEASE INHIBITOR INHIBITING NEUTROPHIL ELASTASE MIGHT DIMINISH NEUTROPHIL-MEDIATED INFLAMMATION

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Trichinella spiralis, a tissue nematode, is an important zoonotic helminth causing trichinosis in human. To survive in their host, the parasite larvae penetrate host skeleton muscles and induce host microenvironment for cyst formation. Additionally, the larvae secrete several biological molecules that exert their roles in host-parasite interaction by modulation and inhibition of host inflammation and immune responses. The serine protease inhibitors commonly found in the larval stage of *T. spiralis* (TsSerp) are suspected secretory proteins. In previous studies, serpins from other pathogens mainly inhibit neutrophil serine proteases [NSPs: Elastase (NE), Proteinase 3 (PR3), and Cathepsin G (CG)] that are crucial mediators in inflammatory responses. However no available data have been suggested for the role of TsSerp in neutrophil-mediated inflammation. In this study, we aimed to elucidate roles of TsSerp in NSPs inhibition and neutrophil-mediated inflammation. Recombinant TsSerp (rTsSerp) was heterologously expressed in M15 strain *E. coli* and purified using Co^{2+} affinity column. The covalent complexes between TsSerp and NSPs were analyzed using reducing SDS-PAGE and western blot. To determine inhibitory activity of TsSerp, specific fluorogenic substrates for NSPs were applied into NSPs/TsSerp reactions and measured an activity with the fluorometer. Our results demonstrated that rTsSerp was successfully expressed in bacteria at molecular weight of 44 kDa. The covalent complex strongly appeared in combination between rTsSerp and NE with dose- and time-dependence but weak when mixing with PR3 and CG. Additionally, rTsSerp could initially inhibit NE to cleave a specific fluorogenic substrate at the enzyme:inhibitor (E:I) ratio of 1:1 and completely inhibit at 1:4. Unfavorably, rTsSerp was not a good inhibitor for PR3 and CG because it could not inhibit their activity even E:I ratio reached to 1:10. These findings suggested that rTsSerp was only specific to NE. Currently, we are determining the inhibitory role of TsSerp in neutrophil-mediated inflammation. We strongly hope that understanding inhibitory roles of TsSerp will benefit drug and vaccine development against trichinosis and might therefore be a valuable therapeutic modality to treat a large spectrum of chronic inflammatory, autoimmune, and allergic diseases in the future.

273

TRICHINOSIS IN ARGENTINA: A 40-YEAR STUDY OF SYMPTOMATOLOGY AND THERAPEUTIC RESPONSE

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Trichinosis is a disease caused by nematodes of the genus trichinella. It is acquired by consumption of undercooked pork meat. *Trichinella* is endemic in certain areas of Argentina, and *T. spiralis* is the most common species. It presents in outbreak fashion, and these can happen any time of the year. In the following study we aimed to characterize the symptoms during different outbreaks of trichinosis and the therapeutic response to different treatments. Retrospective study of 1020 patients with clearly positive epidemiological correlation for trichinosis from the last 40 years at hospital Rawson in Cordoba, Argentina. Patients underwent epidemiologic assessment, physical examination, and complete blood count with differential (CBC). In the last 22 years patients had measurement of creatine-phosphokinase (CPK). Direct immunofluoresce was used for diagnosis of the "Sentinel" case (first presentation of an outbreak), and the rest of the cases associated to each outbreak were based on epidemiological association. Response to therapy was evaluated by improvement of symptoms, CBC and CPK (when applicable). Of the 1020 patients, 89% had typical "toxic symptoms" of trichinosis (headache, weakness, fever), 80% had ocular pain, 78% myalgia, 40% edema of lower extremities, and 43% gastrointestinal symptoms (diarrhea or constipation). Three patients had skin rash and one patient developed meningoencephalitis. 210 patients were treated with thiabendazole. Of these, 64% developed nausea and vomiting as adverse effects (which subsidized upon stopping the drug). Normalization of CBC and symptoms occurred at 4 months in 75% of patients in this group. 90 patients were treated with mebendazole, none of them had adverse effects from the drug. Normalization of CBC and symptoms was achieved at 6 months in 71% of patients in this group. 720 patients were treated with albendazole. There were no significant adverse effects in this group and 90% of patients showed symptomatic recovery at 48hours with normalization of CBC at 45 days. In conclusion, our 4-decade experience with trichinosis in Argentina, we found that the most commons symptoms were the so-called "toxic symptoms", myalgia and ocular pain. These symptoms vary slightly from those reported in the literature, which often includes joint pain, with ocular pain being less common. Therapy with albendazole showed to effective and better tolerated by patients.

274

A GEOHELMINTH SURVEY OF INDIVIDUALS LIVING IN SIX DIFFERENT BRAZILIAN BIOMES: ITS IMPORTANCE IN THE NATIONAL MASS TREATMENT CONTROL PROGRAM

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Soil-borne helminthiasis, geohelminthiasis are infections caused by helminths that undergo part of their life cycle in the soil. The most prevalent are *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms that epidemiologically are typically linked to poverty and underdevelopment. In Brazil the existing data on these infections gives a poor picture of the actual epidemiological situation since it is based principally on old data from hospitals amd health posts. The present study was recommended by the Brazilian Health Ministry to obtain a more up-to-date vision of the epidemiology of geohelminthiasis in the country prior to a mass treatment campaign in association with OMS of 5 to 15 year olds from 719 municipalities. Its aim is to assess the frequency of soil-transmitted helminths in human fecal samples in the campaign´s target group and from co-inhabiting adults living in 35 municipalities belonging to six different biomes: Savana, Pantanal, Amazon Rainforest, Pampa, Caatinga and Atlantic Forest located in five Brazilian regions. The samples are collected from populations that are part of the Family Health Strategy (ESF's) or from basic health units or university hospitals. 200 samples are collected in each of the 35 municipalities totaling 7,000 fecal samples from six biomes. The samples are examined using the Hoffmann sedimentation techniques. Partial results (Pampa biome) show that of the 134 samples analyzed 48.5% were positive for some intestinal parasite. Of this total 26% were positive for helminths. Fecal samples of the Pantanal, part of the Amazon Rainforest and Caatinga are being analyzed. The remaining samples, including the Atlantic Forest and Cerrado will be collected during June and July 2013. The parasitological analysis of all 7,000 samples will be completed by October and the data will be presented in our poster.

275

DEXAMETHASONE INHIBITS MICE BRAIN APOPTOSIS IN EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS* INFECTION

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Angiostrongylus cantonensis, also known as the rat lungworm, is the major cause of eosinophilic meningitis in the Pacific Islands and southeast Asia. Rats serve as the definitive host of the nematode. Humans are infected incidentally and lead to eosinophilic meningitis. Previous mice study had demonstrated increased apoptotic proteins and decreased anti-apoptotic proteins in mice infected with *A. cantonensis*. Steroids have been shown to be one of the effective treatment options for eosinophilic meningitis caused by *A. cantonensis* infection. However, the mechanism of how steroids can influence on eosinophilic meningitis are still unclear. We hypothesize that the beneficial effect of steroid on eosinophilic meningitis are mediated by decrease apoptosis. In a BALB/c mice model, mice were orally infected with 50 *A. cantonensis* L3 via an orogastric tube and were sacrificed every week for 3 consecutive weeks after infection until the end of the study. Dexamethasone was injected via the intra-peritoneal routine from 7th days of infection until the end of the study. Evans blue method was used to measure the blood brain barrier changes and the brain homogenates expression of apoptotic protein and anti-apoptotic protein were analyzed by western blot, immunohistochemistry and TUNEL assay. There was an increased Evans blue amounts, apoptotic proteins (caspase-3, 8, 9 and cytochrome C) and decreased anti-apoptotic proteins (bcl-2) expressions following 2-3 weeks of infection. Dexamethasone administration significantly decreased Evans blue extravasations and apoptotic protein expressions. In conclusion, apoptosis of mice brain homogenates can be repressed by treatment with dexamethasone.

276

FACTORS ASSOCIATED WITH TIMELY IMPLEMENTATION OF MASS-DRUG ADMINISTRATION (MDA) FOR SOIL-TRANSMITTED HELMINTHIASIS (STH)

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More than one billion doses of medicine were shipped by pharmaceutical companies in 2012 for control or elimination of neglected tropical diseases (NTDs). This number is expected to increase to achieve 2020 targets established by the World Health Organization (WHO). Since 2007, Johnson & Johnson has donated Vermox® (mebendazole) for treatment of school-age children to control soil-transmitted helminthiasis (STH). National Ministries of Health or Education request Vermox through Children Without Worms (CWW), which has facilitated drug shipments and provided technical support. CWW reviewed program data on Vermox requests, production, shipments, and treatments for the 11 STH-endemic countries it supported during 2007-2011 to identify factors associated with on-time, delayed or missed mass-drug administration (MDA) campaigns. MDAs were categorized as on time (occurring in or before the planned month), delayed (occurring after but within 6 months of the scheduled month), or missed. Of 58 MDAs for which Vermox was requested and shipped (138,399,000 treatments), 42 (72%) were implemented on time, 8 (14%) were delayed, and 8 were missed. Of the 8 delayed MDAs, 7 occurred within one month of the scheduled date. In-country issues contributed to 6 (75%) of the delayed MDAs. Production, customs, and in-country challenges were some of the issues that contributed to missed MDAs, which occurred in 2008 and 2011, during periods of significant scaling up of the donation. Reported drug coverage rates were comparable between on-time and delayed MDAs (89% vs. 93%, $P > 0.9$). These data suggest that most MDAs are implemented on-time and that missed MDAs are more likely during periods of rapid program expansion. To avoid costs associated with delayed or missed MDAs, careful planning and collaboration are necessary among all stakeholders throughout the supply chain, from production to the community.

277

WATER, SANITATION AND HYGIENE RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INFECTION IN NUEVA SANTA ROSA, GUATEMALA - 2010

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Soil-transmitted helminth (STH) infections cause significant physical and cognitive morbidity. Water, sanitation and hygiene (WASH) likely play roles in STH infection. We conducted a cross-sectional survey of a county in Guatemala to evaluate WASH risk factors for STH infection. We randomly selected households from roofs identified in aerial photos, then surveyed household members and performed environmental WASH evaluations using standardized questionnaires. We used WHO/UNICEF drinking water and sanitation ladders to classify household WASH infrastructure quality as improved or not improved. We tested stool from participants ≥ 1 year of age for STHs using the Mini Parasep® fecal parasite concentrator method and drinking water for *Escherichia coli* using the Colilert® most probable number (MPN) method. We performed unadjusted and multivariable analyses, the latter using the Lasso penalized regression shrinkage method to select variables. We used mixed models to account for household clustering. Models were weighted by the inverse number of roofs per household and adjusted for age category, socioeconomic status, and STH treatment in the past year. We tested 701 persons in 184 households for STH: 76 (11%) 1-4 year olds, 202 (29%) 5-14 year olds, 167 (24%) women of childbearing age 15-44 years old and 256 (37%) other adults ≥ lk15 years old. Most were female (56%), 215 (31%) reported deworming in the past year, and 88 (13%) were positive for *Ascaris*, *Trichuris* or hookworm. Drinking water available for <6 hours/day (prevalence ratio [PR] 4.06, P=0.002), crowding calculated as household members per bedroom (PR 1.24, P=0.049), finished floors (PR 0.25, P<0.001), improved drinking water (PR 0.28, P=0.050) and ever cleaning the sanitation facility (PR 0.33, P=0.026) were significant in multivariable modeling. Our findings support previous studies indicating STH infection is associated with WASH. Basic household interventions to improve safe drinking water availability and behavior changes concerning environmental cleanliness may be helpful in preventing future STH infections.

278

TREAT WORM INFECTIONS WITH CRYSTAL PROTEIN EXPRESSING IN PROBIOTIC LIKE BACTERIA

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Soil-transmitted helminths (namely hookworms, whipworms, and *Ascaris* large roundworms) are intestinal nematodes, which cause diseases of poverty that infect upwards of two billion people worldwide. These parasites are a major threat to health and development of hundreds of millions of children and pregnant women. Enormous hurdles must be overcome in order to develop and deliver urgently needed new therapies

(anthelmintics) to replace old ones that perform sub-optimally and are losing efficacy. Any new therapy must be extremely cheap, be able to be produced in tremendous quantities to treat hundreds of millions of people, have a stable shelf life, and be capable of storage and delivery under adverse environmental conditions. Our research has uncovered a radical and unique new approach that solves each of these challenges: expression of vertebrate-safe, anthelmintic (anti-nematode) proteins in "probiotic-like" food-grade bacteria. Such bacteria can be produced cheaply, in great quantity, stored stably, and delivered under adverse conditions. Here we will discuss our work to develop such engineered bacterial therapy using the anthelmintic crystal protein Cry5B normally made by *Bacillus thuringiensis* (Bt). We will present data on how Cry5B can be expressed in a non-Bt bacterium related to food-grade bacteria, and the strong efficacy of such a bacterium in clearing hookworm infections in rodents. We will also update progress on engineering several food-grade bacteria to express Cry5B as a critical step towards implementation of this novel anthelmintic approach.

279

A QUALITATIVE STUDY EXPLORING BARRIERS RELATED TO USE OF FOOTWEAR IN RURAL HIGHLAND ETHIOPIA: IMPLICATIONS FOR NEGLECTED TROPICAL DISEASE CONTROL

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The role of footwear in protection against a range of Neglected Tropical Diseases (NTDs) is gaining increasing attention Better understanding of the behaviors that influence use of footwear will lead to improved ability to measure shoe use and will be important for those implementing footwear programs. Using the PRECEDE-PROCEED model we assessed social, behavioral, environmental, educational and ecological issues and needs influencing whether and when children wear shoes in a rural highland Ethiopian community endemic for podoconiosis. Information was gathered from 242 respondents using focus groups, semi-structured interviews and extended case studies. Shoe-wearing norms were said to be changing, with going barefoot increasingly seen as 'shameful'. Shoes were thought to confer dignity as well as protection against injury and cold. However, many practical and social barriers prevented the desire to wear shoes from being translated into practice. Limited financial resources meant that people were neither able to purchase more than one pair of shoes to ensure their longevity nor afford shoes of the preferred quality. As a result of this limited access, shoes were typically preserved for special occasions and might not be provided for children until they reached a certain age. While some barriers (for example fit of shoe and fear of labeling through use of a certain type of shoe) may be applicable only to certain diseases, underlying structural level barriers related to poverty (for example price, quality, unsuitability for daily activities and low risk perception) are likely to be relevant to a range of NTDs. Using well established conceptual models of health behavior adoption, we identified several barriers to shoe wearing that are amenable to intervention and which we anticipate will be of benefit to those considering NTD prevention through shoe distribution.

280

INDIVIDUAL CORRELATES OF PODOCONIOSIS IN AREAS OF VARYING ENDEMICITY: A CASE-CONTROL STUDY

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Podoconiosis is a form of non-filarial elephantiasis resulting in bilateral and usually asymmetric lymphedema of the lower legs limited to below the knees. It is common among barefoot people who live in highlands surfaced by red clay soils. In the present study, we aimed to understand the individual correlates associated with podoconiosis by comparing podoconiosis-affected cases and unaffected controls living in areas with three levels of podoconiosis prevalence: 'low' (< 1%), 'medium' (1-5%) and 'high' endemicity (> 5%). Cases (n = 460) and controls (n =707) were recruited from six *kebeles* (the lowest administrative unit) in northern Ethiopia. Data were collected by trained community health workers that identified cases going house-to-house and then by nurses who interviewed identified cases and controls. Cases, especially women, were less educated (OR = 1.73, 95% CI = 1.34 to 2.23, $p < 0.0001$), less likely to be married (OR = 3.43, 95% CI = 2.57- 4.57, $p < 0.0001$) and had lower income ($t = -4.39$, $p < 0.0001$) than controls. There was no statistically significant difference between controls residing in the three areas with varying levels of podoconiosis endemicity. Even though there were no significant differences in foot-washing practices, study subjects with dirty feet were twice (OR = 1.85, 95% CI = 1.36 to 2.51, $p < 0.0001$) as likely to be cases, and those with cracked feet four times (OR = 4.18, 95% CI = 2.738 to 6.370, $p < 0.0001$) more likely to be cases than controls. On average, cases started wearing shoes ten years later than controls ($t = 8.15$, $p < 0.0001$). Among cases, age at first wearing shoes exhibited a positive correlation with age of onset of podoconiosis ($r = 0.63$, $t = 12.51$, $p < 0.0001$). Among all study participants, age at first wearing shoes showed a strong positive correlation with age ($r = 0.81$, $p < 0.001$), and average duration of shoe wearing was less than 30 years, indicating secular increases in shoe wearing over recent years. There was clustering of podoconiosis cases within households, with 142 (30.7%) households containing two or more cases. Based on these findings, we recommend that interventions against podoconiosis adopt household-targeted case tracing. In addition, efforts should be made to bring forward onset of shoe-wearing and to address inequalities in education, income and marriage opportunities, particularly among women.

281

EXTENDING HELMINTH CONTROL BEYOND STH AND SCHISTOSOMIASIS: THE CASE OF HUMAN HYMENOLEPIASIS

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Hymenolepiasis caused by the cyclophyllidean tapeworm *Hymenolepis nana* is the most prevalent human cestodiasis in the world. Similarly to urogenital schistosomiasis, *H. nana* infections can be treated with praziquantel but endemic areas may not overlap. To observe the need to deliver praziquantel to treat *H. nana* infections and investigate the level of overlap with *S. haematobium* infections we used data on *H. nana* infection in children aged ≤15 years collected during an epidemiological survey carried out in northern Angola from May to August 2010. We found that poor sanitary conditions of communities are an important contributor to the high prevalence of *H. nana* infection in the study area. We have also shown that *H. nana* infection is an important contributor to morbidity,

particularly in children aged <5 years. Importantly, our results demonstrate that the spatial distribution of urogenital schistosomiasis and *H. nana* infections differ and distributing praziquantel solely to treat urogenital schistosomiasis will overlook areas with *H. nana* infections. Given the ubiquity and morbidity effects of *H. nana* infections this represents a significant praziquantel gap and advocacy for including this infection in the list of neglected tropical diseases could constitute an important first step towards acknowledging its epidemiological importance.

282

QUANTIFYING THE EXTENT OF MALARIA AND ONCHOCERCIASIS CONTROL ACTIVITIES IN RELATION TO LYMPHATIC FILARIASIS ELIMINATION: A MULTIPLE INTERVENTION SCORE MAP (MISM)

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Lymphatic filariasis (LF) is being targeted for global elimination by 2020, primarily by interrupting transmission through annual mass drug administration (MDA) with either ivermectin or DEC, plus albendazole to the population at risk for at least five consecutive years. Many countries are making good progress, however there is need to better quantify the different levels of MDA coverage, plus other interventions such as ivermectin for onchocerciasis, and vector control for malaria, to determine if high coverage rates of multiple interventions sufficiently overlap high prevalence areas. This study aimed to develop a measure for multiple intervention coverage at both district and sub-district levels. Using Malawi as a case study, we obtained data on MDA coverage (health centre level) from the National LF elimination programme, ivermectin distributions (district level) from onchocerciasis reports, bed net coverage (community level) from multiple Demographic and Health Surveys and indoor residual spraying (IRS) from published literature. Using spatial interpolation methods, coverage levels for MDA and bed nets were smoothed to derive spatially continuous estimates of these measures. Each coverage measure was classified into four levels e.g. for MDA the levels were 0-40%, 40-65%, 65-80%, >80%. Each class was assigned a value between 0 (low) and 3 (high), and these values were summed to produce a single score for each intervention. These intervention scores were combined in a weighted sum, where weights depended on the relative importance of the intervention, and a multiple intervention score map (MISM) was produced. This map highlighted areas in the far north and along the coast of Lake Malawi where consistently high bed net coverage plus IRS may have significantly impacted and/or potentially eliminated LF prevalence, despite lower than average MDA coverage. This multiple intervention score methodology can be easily applied to data from other LF endemic countries, and help guide national LF programmes' future intervention efforts.

283

COMMUNITY PARTICIPATION (CP) ASSESSMENT OF A SCHISTOSOMIASIS CONTROL PROGRAM IN A RURAL AREA OF MINAS GERAIS STATE, BRAZIL

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A schistosomiasis control study was developed using the CP approach in São Pedro a rural community in the municipality of Jequitinhonha Brazil. A modified Rifkin et al. (1988) spidergram was developed for use in São Pedro to measure the extent of CP in five dimensions: 1) leadership of the community and professionals introducing the intervention, 2) planning and management partnerships between the community and professionals, 3) communication, 4) external support, and 5) monitoring and evaluation (M&E) of the participation of intended beneficiaries. Forty-one community leaders and representatives, including local health professionals and district government managers of construction/infrastructure/transportation and municipal health offices participated in the project. The program was developed in three phases, with six meetings each, involving local and district participants and researchers. The participants agreed to improve the water supply/sanitation and build a laboratory (district government managers), attend community meetings, develop a local health community council (community representatives), and provide health education, diagnostic and schistosomiasis treatment services (researchers). All designated actions except the construction of a laboratory and a sewage treatment system were completed. Preliminary spidergram assessment indicates that whereas CP in leadership, planning and management, and community was satisfactory, scoring 3 each on a five point scale, external support and M&E lagged behind (scoring 2 points each). Moreover, while attendance of meetings was overall 60%, it declined after initial high attendance rates and male participation was low throughout the two-years of the project. The results indicate that the spidergram method can measure CP in schistosomiasis prevention and control. However, the program needs to strengthen community capacity to sustain the intervention, improve feedback to the community and facilitate participation in data collection and M&E.

284

HOW THE PRIVATE SECTOR HAS MADE A DIFFERENCE IN NEGLECTED TROPICAL DISEASES CONTROL PROGRAMS

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The fact that the term 'neglected tropical diseases' (NTDs) was first used in 2004/2005 to describe parasitic and bacterial diseases that affected the poorest of the poor, and that the global movement to reduce the burden of these diseases has significantly intensified with multimillion dollar grants, illustrates that the global health community has prioritized the need to control these diseases. The movement significantly intensified in January 2012 when the London Declaration announced unprecedented commitments to support the WHO's goal of controlling and eliminating 10 of the NTDs by 2020. Even as the global movement to end NTDs continues to gain momentum, these diseases continue to inflict suffering and chronic disability on 1.5 billion of the world's most impoverished. In an effort to mobilize the necessary funding to expand control efforts and reach the 2020 NTD goals, the private sector has actively intensified its support. The END Fund, the world's first private initiative aimed at tackling NTD was launched in June 2012. Since launching, the END Fund has progressively expanded support efforts and is now working in 13 countries. When Mali's funding from USAID was frozen due to the 2012 coup d'etat, the END Fund was able to mobilize the necessary US$1.2m to re-launch the country's mass treatment campaign. By identifying a group of private sector donors, including gold mining companies and private foundations, the END Fund ensured Mali's NTD control program did not lose the impressive gains made in the previous years to reduce the NTD burden throughout the country. The END Fund and its private sector investors are committed to improving the health of those affected by NTDs. By establishing multidisciplinary partnerships, the END Fund brings new donors to the NTD movement and invests the much needed funding into control programs. By helping to treat communities and improving the health and well being of societies as a whole, the END Fund is contributing to breaking the poverty cycle that NTDs force upon the world's most impoverished.

285

DOES CLINICAL ACTIVITY PREDICT CHLAMYDIAL INFECTION AFTER MASS ANTIBIOTIC TREATMENTS FOR TRACHOMA?

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Global efforts to eliminate blinding trachoma target the ocular strains of *Chlamydia* trachomatis that cause the disease. Mass antibiotic distributions are a major strategy for trachoma elimination. Testing for chlamydial infection by PCR is expensive, so clinical examination is widely used to determine whether or not mass antibiotic distributions are necessary. Previous studies reveal a relatively high correlation between clinical activity (TF or TI utilizing the WHO grading system) and chlamydial infection before mass treatment. Unfortunately, this correlation decreases dramatically after mass treatment--it is difficult to determine who is infected by clinical exam after treatment. However, treatment is at the community level. Here, we assess whether the prevalence of clinically active trachoma predicts chlamydial infection at the community level, using longitudinal results from a cluster-randomized controlled trial of mass azithromycin distributions in Niger. 48 Nigerien communities were randomized to annual or biannual treatment. Every six months, a random sample of up to 100 children (0 - 5 years of age) in each of the 48 communities were examined for trachoma, and conjunctival swabs were collected for chlamydial testing with PCR. The mean antibiotic coverage was 88.2% per community, with a mean of 89 children examined and 168 children treated. At baseline, the correlation between the prevalence of clinical trachoma and chlamydial infection was 0.64 (95% CI: 0.38 to 0.79) and by 24 months, 0.47 (95% CI: 0.23 to 0.70). At 24 months, we were unable to demonstrate that the correlation coefficient was lower than that at baseline (p = 0.42). Most trachoma elimination programs utilize clinical examination to determine their mass antibiotic strategy due to the high costs and resources associated with PCR processing to test for chlamydial infection. While clinical exam has not been shown to be useful at the individual level, this study suggests that clinical activity and chlamydial infection are correlated, even after mass antibiotic treatment. We were unable to find a significant difference in the correlation at baseline and 24 months.

286

ERADICATION INVESTMENT CASE FOR ONCHOCERCIASIS

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The health and economic benefits of disease eradication may be substantial, but costs and consequences should be carefully evaluated. An Eradication Investment Case (EIC) is an analytic-deliberative process to support decisions on whether to launch eradication initiatives. The EIC for onchocerciasis was developed adopting an approach, proposed at the Ernst Strüngemann Forum 2010, including feasibility assessment, the estimates of costs, and the health and economic impacts. Strategies to achieve elimination and eradication were developed as scenarios. The number of treatments, the financial and economic costs of delivering them, and the public health and economic impacts were assessed for the time horizon 2013-2040. Costs were estimated using a micro-costing approach, while the public health impact was estimated with a micro simulation model. Geographical scaling up of treatment coverage to achieve elimination in African countries would require 48 million treatments annually on average, less than a half of the annual treatments needed, if current control levels are maintained through 2040. Accordingly, the annual financial cost for 2013-2040 will decrease from $23 million to $10 million as the goal shifts from control to elimination or eradication. Scaling up and sustaining the treatment coverage for onchocerciasis elimination and eradication is also predicted to lead to substantial economic benefits in the long run, which are only marginally reduced by the increase in surveillance costs to sustain elimination, mainly due to direct and indirect cost savings of averting skin-itch cases. Marginal health benefits of onchocerciasis elimination versus control are more limited, since the current control strategies already reached high treatment coverage in areas where onchocerciasis is considered to be a public health problem; and non-covered areas mainly concern low endemic areas. The EICs shows that a goal shift to elimination and eradication of onchocerciasis is predicted to generate substantial future economic benefits.

287

MEDICAL STUDENT KNOWLEDGE OF NEGLECTED TROPICAL DISEASES IN PERU

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Education to health-care professionals is a cornerstone in the battle against neglected tropical diseases (NTD) in developing countries. Few studies have evaluated the level of knowledge of undergraduate medical students in clinical and epidemiological aspects of NTD. The aim of this study was to describe the level of knowledge in NTD among medical students from a private school of medicine in Lima, Peru. A twenty-multiple choice questionnaire was given to students from first to seventh year (last year) of medical school of Universidad Peruana Cayetano Heredia in Lima, Peru; to be completed confidentially in 10 minutes. The questionnaire was composed by two blocks of questions. Block I consisted of epidemiological questions and block II of clinical vignettes. We arbitrarily defined knowledge as low or suboptimal (≤ 30% correct answers), moderate or acceptable (31-69%) and high or outstanding (≥70%). A total of 586 students (49.3% female, 50.7% male; age mean ± SD = 21.3 ± 2.4 years) completed the voluntary and anonymous survey. The prevalence of high-score grades of knowledge increased significantly from first-year (1.9%) to senior medical students (57.9%) (p=0.0001). Higher outstanding scores were found in clinical (85.9%) compared to epidemiological knowledge (21%) in last-year medical students (p=0.0001). In contrast, lower scores (suboptimal) were more prevalent in epidemiological (24%) than clinical knowledge (5.3%) in senior medical students (p=0.01), which indicates that clinical knowledge improves towards the end of career whereas epidemiological concepts remain low. In conclusion, students from this medical school in Peru have an outstanding knowledge about NTD at the end of the career mainly in clinical concepts, but acceptable or suboptimal in the epidemiological area. These results stress the importance of intensifying the education in epidemiology of NTD in undergraduate medical students at developing countries.

288

LARGE-SCALE INTEGRATION OF PUBLIC HEALTH ACTIVITIES FOR TROPICAL DISEASES IN TOGO

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Togo, a country in west Africa, is endemic for several neglected tropical diseases, including onchocerciasis, schistosomiasis, and soil-transmitted helminthes (STH). Togo has been a pioneer in integrating public health activities for tropical diseases, beginning as early as 2004. The first nationwide mass drug administration (MDA) occurred in August 2011, and included not only treatment for onchocerciasis, schistosomiasis, and STH for persons five years old and older, but also vitamin A and STH treatment for children under 5 and insecticide treated bed net distribution for each household. The 2011 integrated MDA involved many partners, including different sections of the Togo Ministry of Health as well as external partners including the Global Fund, USAID, Health and Development International, UNICEF, Red Cross, Vestergaard, World Health Organization, Plan Togo, OCDI, and Sightsavers. The MDA was implemented by over 5,600 community drug distributors and took place in three phases. The first phase involved a census, vitamin A supplementation, albendazole treatment based on local STH prevalence, and ivermectin treatment based on local onchocerciasis prevalence. The second phase included distribution of coupons for insecticide treated bed nets for each household and administration of praziquantel based on local schistosomiasis prevalence. The third phase of the 2011 MDA involved distribution of the bed nets. Ultimately, the integrated MDA of 2011 treated 1.3 million children for schistosomiasis, 1.8 million children for soil-transmitted helminthes, and 2.4 million people for onchocerciasis, supplemented over 900,000 children with Vitamin A, and distributed over 2.5 million bed nets. These activities were possible in 2011 only through the efficiency of large-scale integration across programs. Integrated activities, however, are more complex and require greater coordination at all levels of the health system in order to succeed. The strong leadership at the Ministry of Health promoted an unprecedented degree of partnership and sharing of resources among the different organizations, making this integrated campaign a success.

289

FACTORS UNDERLYING THE SUCCESSFUL LARGE-SCALE INTEGRATION OF PUBLIC HEALTH ACTIVITIES FOR TROPICAL DISEASES IN TOGO

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Togo has a long history of integrated health activities for control of tropical diseases, beginning as early as 2004. The first nationwide mass-drug administration occurred in 2011, and included not only integrated treatment of onchocerciasis, schistosomiasis and soil-transmitted helminthes, but also vitamin A supplementation and insecticide treated bed net distribution for each household. This large-scale integration of public health activities was made possible by strong leadership from the Togo Ministry of Health (MOH), which coordinated the shared training, logistical, and supervisory activities. Four committees, each of which included representatives from each partner organization, organized the integrated activities. The Technical Committee developed the shared training materials and reporting tools and organized successive trainings of the district-level supervisors, followed by the sub-district-level supervisors, and finally the community drug distributors. The Logistics Committee was responsible for ensuring all of the training materials, reporting forms, registers, bed net coupons, drugs, vitamin A, and insecticide-treated bed nets were distributed across the country. The Social Mobilization Committee was responsible for informing the public about the upcoming integrated public health activities and generating public service announcements. Finally, the Finance Committee was in charge of the integrated budgets and ensuring that there were sufficient resources for all of the integrated activities. Although many challenges were faced, the Togo MOH managed to lead all of the Committees to a successful integrated activity in August 2011. Overall, the integration of albendazole, ivermectin, and praziquantel treatment, vitamin A supplementation, and bed net distribution demonstrated the efficiency of an integrated approach. Each of the partners contributed to the shared activities, which resulted in more people receiving greater public health benefits than would have otherwise been possible.

290

INTERACTIONS BETWEEN COENZYME Q10, MELARSOPROL AND *TRYPANOSOMA BRUCEI RHODESIENSE* INFECTION MODULATE ANTI-OXIDANT DYNAMICS MOUSE MODEL

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Melarsoprol (Mel B) is the only efficacious drug against Late stage Human African Trypanosomiasis (HAT), but inadvertently induces Post Treatment Reactive Encephalopathy (PTRE) and unacceptable mortality of 5% among patients. Investigations were conducted to establish neuro-protective role of specific antioxidants (Manganese Superoxide dismutase (MnSOD), Glutathione Reductase (GR), Copper-Zinc Superoxide dismutase (SOD-1) and reduced glutathione (GSH) and Coenzyme Q10 CoQ10) against PTRE and putative resultant brain degeneration in a mouse model. Additionally, the role of antioxidant capacity in neurodegeneration due to *Trypanosoma brucei rhodesiense* was investigated. Female Swiss-white mice were infected with *T. brucei rhodesiense* parasite and were manipulated to simulate all phases of HAT and PTRE. Expression profiles of the antioxidants in brain tissues were assessed using immunoblot or spectrophotometric procedures. There were significantly higher expressions of MnSOD (P=0.0014), SOD-1 (P=0.0001), and GR (P=0.0083) in infected than uninfected mice 21 days post infection (dpi), which were two-folds lower than those observed at 57 dpi. Levels of GSH were significantly lower (P=0.0347) in infected 57dpi than uninfected mice 21dpi. Expressions of SOD (P=0.0429), GR (P=0.0001) and GSH (P=0.0001) were significantly higher in infected than in uninfected mice among Mel B treated mice. Mel B treated uninfected mice had significantly lower expressions of GR (P=0.0001) and GSH (P=0.0001) and MnSOD (P=0.0035), than untreated (uninfected) controls. Pre-treatment with CoQ10 significantly increased expression of GSH (P=0.0001) relative to the Mel B treatment alone. The results indicate that the parasites and Mel B suppress the antioxidant system, while CoQ10 appears to restore it. The time dependent dynamics of antioxidant suppression due to Mel B, and potential ameliorating effects of CoQ10 on the same, indicate putative mechanism underlying and antidote to the toxicity of the drug with potential application in formulation of novel Mel B based drugs and development of novel markers for staging the disease.

291

PROTEOMIC APPROACH FOR THE IDENTIFICATION OF NOVEL MARKERS FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is an infection transmitted by phlebotomine flies and caused by parasite of the genus *Leishmania*. This disease is characterised by fever, weight loss, enlargement of the liver, spleen and

lymph nodes and low blood cell count. Early and accurate laboratory diagnosis is essential before initiating treatment, for clinical outcome reasons. The clinical features of VL resembles those of several other disease including malaria, tuberculosis etc, effective drug are available but they need to be administered for a minimum 3 weeks and are potentially toxic and expensive. For diagnosis of VL, rK39 antigen based rapid test is widely used. Unfortunately, up to 32 % healthy individuals from endemic region test positive with this antigen in Indian subcontinent. There is an urgent need to search for a more specific antigen with more precise specificity but sensitivity similar to rK39 antigen. We identified *Leishmania donovani* specific 70kDa (BHUP1), 37kDa (BHUP2), 12.6kDa (BHUP3) soluble promastigote antigen through western blot technique, in order to develop a diagnostic marker for VL. On blotting, antibody against this protein was recognized by all VL patient's sera, but, it was absent in every control group non-endemic healthy control (NEHC), endemic healthy control (EHC) and different disease (DD). The diagnostic potential was further validated by ELISA using serum of VL, NEHC, EHC and DDs. The Sensitivity of the ELISA were found to be for VL (96, 95, 88%), whereas the specificity ranges for EHC (96, 98 and 96 %), NEHC (100% for three antigen) and DD (97, 97 and 97.4 %) groups respectively, against BHUP1, BHUP2, BHUP3 antigen respectively. Furthermore, it was characterized by 2D-PAGE analysis followed by MALDI-TOF analysis. Due to excellent sensitivity and specificity of these antigens, it warrants the further development as a tool for diagnosis of VL.

292

HIGH-THROUGHPUT IN VITRO SCREENING OF SELECTED GHANAIAN MEDICINAL PLANTS FOR TRYPANOCIDAL ACTIVITY AGAINST T. BRUCEI

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Human African Trypanosomiasis (HAT) is a devastating disease in Africa. Available drugs are characterized with unpleasant limitations. This has necessitated the development of alternate treatment for trypanosomiasis. Ghana has a long history on use of traditional medicines which have not been scientifically evaluated. This is needed to establish any as new chemotherapy for trypanosomiasis. Thus, our aim was to establish a high-throughput in vitro screening system for trypanocidal drug candidates among selected Ghanaian medicinal plants (GMP). Crude extracts from selected GMP were screened for trypanocidal activity against the GUTat 3.1 strain of T. brucei using a 3-step in vitro system. Effects of the extracts on T. brucei viability and proliferation were determined by Alamar blue® assay (Invitrogen). Subsequently parasites were subjected to FACS analysis to investigate extracts' ability to induce apoptosis and/or cell cycle alteration. Finally, immunohistochemistry was used to detect any extract-induced morphological and marker expression changes in parasites. Crude extracts with activity in the first round 3-step screening were fractionated and re-screened. Further purification of active fractions into compounds was then done. The purified compounds were also taken through this 3-step screening for identification of active compounds. Out of 113 crude extracts screened, 8 showed strong trypanocidal activities and induced apoptosis. In addition, some extracts showed G2/M phase alteration during cell cycle in trypanosomes. Upon further analysis, a total of 4 including 2 novel active compounds have been identified. These caused flagellum deficiency in the parasites. This 3-step screening system enabled us to follow the interesting molecular activities of active components at various fractionation and purification stages. In vivo studies for efficacy and safety of the active compounds are ongoing. Our high throughput screening system successfully identified molecular mechanisms such as apoptosis and cell cycle alterations in the parasites.

293

MOLECULAR TARGETS AND ANTILEISHMANIAL DRUG LEADS IDENTIFIED THROUGH SCREENING A LIBRARY OF NATURAL PRODUCTS PREPARED BY HIGH THROUGHPUT FRACTIONATION PARADIGM

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Visceral leishmaniasis, caused due to infection of *Leishmania donovani*, is a prominent disease in certain tropical regions of the world. Toxicity and suboptimal efficacy of current antileishmanial drugs necessitate the discovery of new antileishmanial drugs with better efficacy and safety profiles. Natural products remain an unmatched source of drugs with novel chemotypes. However conventional screening of crude natural products extracts suffers from certain limitations. Polyphenols, can cause false-positive results in *in vitro* screening. The presence of chemical diversity found a single extract. Biologically active compounds present at extremely low concentrations in crude extracts. Pre-fractionation technologies have been useful in overcoming these limitations. A library of >30,000 natural product fractions have been generated through a high throughput fractionation paradigm. Each fractions, in 96 well plates, have been , analyzed via QC-UPLC MS/MS. 7387 fractions from 671 plant extracts were screened through newly developed *L. donovani* Macrophage (THP1 cells) amastigotes parasite rescue and transformation assay and for cytotoxicity against differentiated THP1 cells. A total of 194 fractions were identified with 50% or higher inhibition vs. leishmania amastigote growth in THP1 cells; only 10 of these were toxic against the THP1 cells alone. The active 194 fractions were further screened for dose-response antileishmanial analysis. The selective antileishmanial activity of 65 fractions was confirmed. Active fractions from *Thuja occidentalis* (IC50-0.25 mg/mL), *Asclepias asperula* (IC_{50}-0.33 mg/mL), *Rhodea japonica* (IC_{50}-0.41mg/mL) and *Nerium oleander* (IC_{50}-0.03 mg/mL) were selected for further investigations. Deoxypodophyllotoxin from *T. occidentalis*, oleandrin from *N. oleander*, rhodexoside from *R, japonica* and a cardinolide from *A. asperula* were identified as actives, and potential new antileishmanial drug leads. These diverse compounds, which are potential inhibitors of DNA Topiosmerases and Na+,K+-ATPases, were selectively active against intracellular amastigotes with so significant effects on leishmania promastigotes. The results indicate Na,K-ATPase as potential new molecular drug target and confirm topisomerase as the promising target for new antileishmanial drug discovery.

294

EFFECT OF FE, ZN AND CU IN VITRO PRODUCTION OF IFN-Γ, IL-13 BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENTS WITH CUTANEOUS LEISHMANIASIS WITH TREATMENT FAILURE IN BOLIVIA

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Resistance to pentavalent antimonial(SBV) agents as Glucantime, is creating a major problem in the treatment of cutaneous leishmaniasis (CL). Generate new therapeutic approaches in the treatment regimen that could reverse antimony resistance is a public health problem in Bolivia. An appropriate balance between pro-inflammatory and anti-inflammatory cytokines that mediate innate and adaptative immune responses is required for effective protection against human leishmaniasis.

The study evaluated the effect of Fe, Zn and Cu as an alternative in the specific treatment and its influence on the immune balance in patients with CL with therapeutic failure (RESISTANT) and patients who responded successfully to treatment (SENSITIVE) enrolled as cases-controls respectively. Peripheral blood mononuclear cells (PBMC) were stimulated *in vitro* with soluble antigen proteins of leishmania *sp.*, to determine T-cells responses in cell culture medium supplemented with Fe, Zn and Cu. Anti *Leishmania* humoral and cellular immune response was evaluated by: The production of INF-γ and IL-13, as markers of Th1 and Th2 response respectively. Cellular response to the superantigen SEB were also monitored in this study. The data obtained indicate that PBMC of sensitive and resistant patients displayed an increased production of IFN-γ in absence of these trace elements that are associated with the low production of IFN-γ. This was associated with a type 1-biased immune environment, since cells of Resistant and Sensitive groups produced higher INF-γ levels in response to SEB. Resistant patients produce more IFN-γ in response to leishmania Ag that sensitive patients. IL-13 production remained low and similar in the two study groups, whatever the condition of stimulation. Our data indicated that these trace elements may influence the balance of TH1 cytokine production and possible therapeutic administration in parallel with the specific treatment should be evaluated, since the production of IFN-γ significantly decrease by effect of Fe, Zn and Cu and might augment susceptibility to infection.

295

PARASITE PERSISTANCE AND CLINICAL RESPONSE TO MILTEFOSINE IN PATIENTS WITH CUTANEOUS LEISHMANIASIS

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Miltefosine (MLF) is an oral drug approved for the treatment of leishmaniasis in adults and children >2 years of age. Knowledge of parasitic response to treatment with miltefosine is limited. Previous studies have demonstrated persistence of parasites in patients with treatment failure and case reports have shown decrease in parasite load after treatment in patients with clinical cure.This study aimed to determine the parasitologic response to MLF during and after treatment of cutaneous leishmaniasis caused by species of the *Viannia* subgenus, and its relationship with the therapeutic outcome. A sample size of sixty was estimated for this study based on population pharmacokinetic modeling. Participants received supervised treatment with MLF (1.8-2.5 mg/kg/day, during 28 days). Clinical response was evaluated at the end of treatment, and 13 and 26 weeks after initiation of treatment. Presence of *Leishmania* was determined by kDNA PCR in aspirates and swabs of lesions/scars obtained pre-treatment, days 15 and 29, and 13 weeks after beginning treatment. To date 49 patients, 22 children (2 - 12 years old) and 27 adults (18-60 years old) have been enrolled; 26 patients have completed follow up with a final cure rate of 100% (n=15) in adults and 81.8% (n=11) in children. Apparent cure was observed in 6.8% (3/44) at the end of treatment and in 54% (20/37) at week 13. Pre-treatment lesion samples from all patients were positive for kDNA. At day 15, 27% (10/36) and post-treatment 23% (9/39) of patients were kDNA positive. Thirty percent (6/20) patients with apparent cure at week 13 were kDNA positive. In conclusion, these initial observations confirm the persistence of parasites following clinical cure in response to miltefosine treatment, and raise concerns regarding the potential for emergence of drug tolerant populations.

296

TREATMENT OF BOLIVIAN CUTANEOUS LEISHMANIASIS WITH INTRALESIONAL DRUGS

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Cutaneous leishmaniasis (CL) is a disease that ultimately self-cures. Whether local or systemic therapy should be used to treat this disease is presently undecided. Factors favoring systemic therapy are high cure rate and theoreticaly protection against mucosal dissemination. Factors favoring local therapy are the presence of only 1 lesion, the rarity of mucosal dissemination which systemic CL therapy might prevent, and adverse effects of systemic drugs. We have reported a 6-month cure rate of 70% in 30 patients with single Bolivian CL lesions treated with 3 intralesional injections of pentavalent antimony. When failure did occur, it was seen at the 1 month and 3 month follow up periods. There was no relapse at 6 months of previously cured disease. Adverse effects were limited to mild injection site pain. In comparison, a placebo group of 30 patients demonstrated a 17% cure rate. A cryotherapy group of 20 patients had a surprisingly low cure rate of 20%. To attempt to increase the cure rate yet not overly burden patients with clinic visits, we are now evaluating 5 intralesional injections of pentavalent antimony and also intralesional injection of other antileishmanial agents. Efficacy at intermediate and 6 month follow up periods in the present study will be presented in comparison to that of the active and placebo groups of the prior study. The possible adverse effects of pain, itching, inflammation, and vesicular formation in the present study will also be presented in comparison to the values for the active and placebo groups of the prior study. An overall evaluation of efficacy, tolerance, and feasibility (a particular issue for repeated physical interventions such as topical application of drugs) for local measures vs systemic treatments will be presented.

297

A RETROSPECTIVE ANALYSIS OF 400 CASES TREATED FOR VISCERAL LEISHMANIASIS IN GEORGIA

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Visceral Leishmaniasis (VL) is one of the most widespread zoonotic infections in Georgia. The causative agent of VL is L. infantum. It mostly affects children under 5 years old. In recent years, the number of new cases among adult population has increased. HIV/Leishmaniasis co-infection has also increased during the last several years, which is characterized with increased resistance to treatment and high rates of relapse. We studied the medical records of 400 patients with visceral Leishmaniasis who were diagnosed and treated between 2009 and 2012. Among these 400 patients, 300 were treated with Meglumine Antimoniate (Glucantim) and 100 were treated with Lyposomal Amphotericin B (Ambisome). The dosage of Glucantime constituted 20 mg/kg and summary dosage of Ambisome constituted 20-30 mg/kg. Among those treated with Glucantim 292 (97.3%) patients were cured and in 8 (2.7%) patients the treatment was stopped. Among these 8 patients 5 died. In 3 cases the cause of death was liver failure and in 2 cases severe arrhythmia due to toxic side effects of Glucantime. In the rest of patients Glucantime treatment was stopped due to drug induced liver failure or cardiac arrhythmia. Side effects of Glucantim treatment was observed in 39 (13%) patients. Relapse after Glucantim treatment

was observed in 14 (4.6%) cases. Among those who were treated with Ambisome (100 patients) complete course of treatment was received by 97 (97%) of patients and in 3 (3%) cases the treatment was interrupted because of lethal outcome in 2 cases and allergic reaction in 1 case. Side effects of Ambisome treatment was observed in 7 (7%) patients. Among 12 study participants who had HIV/*Leishmania* co-infection 9 (75%) patients developed relapse and 3 (25%) patients died. Relapse occurred after the treatment both with Glucantime and Ambisome. All HIV infected study participants had more than 2 co-infections and CD4+ count with < 200 cell/μL. Relapse occurred even after increasing the dose of Ambisome to 30 mg. Both anti-leishmania drugs (Glucantime and Ambisome) manifested the high clinical effectiveness. The effectiveness of both drugs in patients with HIV/*Leishmania* co-infection was very low.

298

IN VITRO SCREENING OF COMPOUNDS AGAINST *LEISHMANIA AMAZONENSIS* AMASTIGOTES

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There is an estimated burden of 12 million infections by *Leishmania* worldwide. Without an effective vaccine and few drugs, there is a need for new treatment alternatives. Despite advances in parasite biology and genetics, development of new compounds is limited. High Throughput Screening of compounds with methods closely mimicking natural mammalian infection brings advantages to new drug development. We performed a pilot *in vitro* screen of 2400 compounds at 10 μM using the DIVERSet Library (Chembridge Corporation) on wild-type *L. amazonensis* axenic amastigotes measuring cell viability via alamarBlue® fluorescence. Selecting hits with at least 75% activity with respect to Pentamidine control yielded 53 compounds (2.2%). The assay had good signal to background ratio, with a five-fold difference between controls and Z-scores ranging from 0.52 to 0.63. Hits were then tested for efficacy against infected J774 macrophages with a strain of *L. amazonensis* expressing β-lactamase. Ten compounds (18%) had significant activity against intra-macrophage amastigotes at 10 μM, but with lower efficacy compared to axenic amastigotes. J774 cell toxicity curves showed that 22% of compounds were not toxic at a concentration of 200 μM, 55% were toxic at 200 μM but had no effect at 10 μM while 23% had toxicity below 10 μM. Thus, we have selected 6 hits with high intra-macrophage efficacy and low toxicity to host cells. The results obtained from this pilot screening assay with *L. amazonensis* amastigotes show good reproducibility and reliability, validating this assay for high throughput screening. Furthermore, the intracellular and toxicity assays enable a better narrowing down of compounds for future *in vivo* assays to assess clinical efficacy.

300

STUDIES ON THE MECHANISM OF ACTION OF THE ARYLIMIDAMIDE DB766 IN *LEISHMANIA DONOVANI*: ROLE OF CYP5122A1 AND AZOLE INTERACTIONS

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Arylimidamides (AIAs) show potent activity against intracellular parasites. The frontrunner AIA DB766 (2,5-bis[2-(2-*i*-propoxy)-4-(2-pyridylimino) aminophenyl]furan hydrochloride) displays potency similar to amphotericin B against intracellular *Leishmania* amastigotes and is orally active in animal models of visceral leishmaniasis, but its mechanism of action is unknown. Ultrastructural studies in *L. donovani* exposed to DB766 revealed effects similar to those observed in the presence of sterol biosynthesis inhibitors, such as the appearance of vesicles in the cytoplasm, flagellar pocket, and flagellum. *L. donovani* axenic amastigotes were also raised that displayed 12-fold resistance to DB766. These DB766 resistant parasites (DB766R) were not cross resistant to pentamidine, but were hypersensitive to the sterol 14α-demethylase (CYP51) inhibitors ketoconazole and posaconazole (2000-fold more sensitive and over 12,000-fold more sensitive than wild type, respectively). The expression of CYP5122A1, a recently identified cytochrome P450 associated with ergosterol metabolism in *Leishmania,* is dramatically reduced in the resistant parasites as assessed by western blotting. Consistent with these observations in DB766R parasites, CYP5122A1 half knockout *L. donovani* promastigotes were significantly more susceptible to ketoconazole and less sensitive to DB766 than their wild type counterparts. Synergistic activity was also observed in both *L. donovani* axenic amastigotes and intracellular forms with DB766-posaconazole combinations. These studies form the basis for mechanistic hypotheses concerning the antileishmanial action of the AIA DB766 and indicate that DB766-azole combinations may hold promise for the treatment of leishmaniasis.

301

DISCOVERY AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 2-CHLORO-N-(1-HYDROXY-1,3-DIHYDROBENZO[C][1,2]OXABOROL-6-YL)-4-(1H-PYRAZOL-1-YL)BENZAMIDE (AN7973) FOR THE TREATMENT OF AFRICAN ANIMAL TRYPANOSOMIASIS

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Animal African Trypanosomiasis (AAT) is a parasitic disease caused by tsetse fly-transmitted trypanosomes that include *Trypanosoma congolense, T. vivax, T. brucei brucei*. AAT results in serious economic losses in livestock because of reduced productivity, variable clinical signs (e.g. anemia, emaciation) and death. Drug resistance has been an increasing problem in chemotherapy and chemoprophylaxis control. Leveraging the experience of the successful development of a clinical drug candidate against Human African Trypanosomiasis (HAT), we have screened the Anacor benzoxaborole compound library against *T. congolense* (IL-3000) in a 3-day *in vitro* cell viability assay, in order to identify novel chemical entities for AAT compared to the current standard drugs, diminazene and isometamidium. A number of benzoxaborolyl-benzamide analogs were found to show IC_{50} values of less than 100 nM including AN7973, which showed an IC_{50} value of 57 nM. Compounds bearing hydrophobic groups, such as chloro and trifluoromethyl, tended to show potent activity. In contrast, polar substituents, such as carboxy, were not tolerated. Since many compounds showed almost equally potent activity *in vitro*, compounds to be tested *in vivo* were chosen based on the efficacy data against *T. b. brucei* in mice that had been obtained through the HAT study. The selected compounds were tested for their efficacy in an *in vivo* mouse model against *T. congolense* (STIB736/IL1180). Several compounds showed 100% cure lasting > 60 days by intraperitoneal administration at 10 mg/kg for 4 days. AN7973 showed 100% cure at a lower dose of 3 mg/kg for 4 days as well. AN7973 showed potent activity against *T. congolense* both *in vitro* and *in vivo* and appears to be a promising lead for an anti-AAT agent.

302

LEISHMANIA BRAZILIENSIS RECOMBINANT HISTONE H2B FOR THE SERODIAGNOSIS OF TEGUMENTARY LEISHMANIASIS

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American tegumentary leishmaniasis is an important endemic disease in Peru and other countries in the New World. It presents a spectrum of clinical manifestations, the principal forms being cutaneous (CL) and mucocutaneous leishmaniasis (MCL). At present, there is not an ideal diagnosis method that matches quality (sensitivity and specificity) and affordable cost for field use. Parasitological diagnosis by microscopy and culture has low sensitivity while DNA-based diagnosis (PCR) is highly sensitive but expensive and technically cumbersome. Hence, a method relying on serological diagnosis is desirable. While serological assays have proved useful for diagnosing visceral leishmaniasis cases which have a marked humoral immune response, there is not such a standardized method available for diagnosing CL and MCL cases. Typically total crude leishmanial antigen is used with variable sensitivities and with cases of cross-reaction. A good candidate for serological diagnosis reported from the Old World is the histone H2B. We hypothesize that serological detection of a recombinant H2B from *Leishmania braziliensis*, the most prevalent species in Peru, would simplify diagnosis, reduce cross-reaction and ultimately allow broad standardization of the method for its use in endemic regions. To this end, the L. *braziliensis* h2b gene was cloned in pET-21a(+) vector and expressed in *E. coli* BL21(DE3) by induction with 1mM IPTG during five hours at 37°C with shaking. Bacteria were lysed and the recombinant protein was purified by nickel affinity chromatography. Serum samples from patients originating from an endemic region were pre-absorbed with *E. coli* lysate and assayed for reactivity by western blot. We have assayed two CL and three MCL patients and found reactivity for the five samples, with apparently higher titers in the MCL form. We did not find cross-reaction with serum from an acute *Trypanosoma cruzi*-infected patient and with a non-endemic negative control donor. Currently, we are analyzing a bigger set of samples, the results of which will be shown at the meeting. In the future we expect that a serological assay based on this protein can be used in the field for epidemiological surveillance of tegumentary leishmaniasis.

303

LIPOSOMAL RESIQUIMOD FOR THE TREATMENT OF *LEISHMANIA DONOVANI* INFECTION

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Leishmania is a zoonotic parasite that causes Leishmaniasis; a disease that affects approximately 12 million people worldwide. Leishmaniasis occurs either in its cutaneous (CL) or visceral (VL) form. VL is responsible for the deaths of roughly 50,000 people per year. Treatments exist to treat VL, including intravenous (i.v) delivery of antimony or liposomal amphotericin B, and recently developed oral formulations, such as Miltefosine. While these treatments have been effective, resistant strains can be a problem and require constant development of novel treatment formulations, including those that could potentially eliminate infections through immune-mediated mechanisms. Imidazoquinolines, a family of immunomodulatory toll-like receptor 7/8 agonist compounds, have been quite successful at treating CL, however they have not been utilized for parenteral delivery due to their hydrophobicity. One way to deliver a hydrophobic compound systemically is by incorporation into a drug delivery vehicle, such as a liposome. Using a lipid film hydration method, followed by extrusion, we were able to encapsulate the imidazoquinoline resiquimod and deliver the compound by i.v injection to *L. donovani* infected BALB/c mice. Liposomal resiquimod significantly decreased parasite load in the liver, spleen, and bone marrow while simultaneously stimulating interferon-γ and interlukin-10 production. Histological and *in vitro* analysis showed liposomal resiquimod was non-toxic. Liposomal delivery of FDA-approved resiquimod provides a promising avenue for immune mediated clearance of VL infections.

304

CHARACTERIZATION OF DEVELOPMENTAL STAGE AND STRAIN SPECIFIC SALIVARY ANTIGENS OF *TRIATOMA INFESTANS* AS POTENTIAL EXPOSURE MARKERS

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Antibody responses of animals to saliva of *Triatoma infestans* can be useful indicators of the spatial distribution of triatomines and therefore they could support vector control efforts in endemic Chagas disease areas. As previously presented, salivary protein profiles of *T. infestans* differ among development stages and strains, and due to these differences host antibody responses can also vary. These differences should be taken into account when screening for a potential marker of triatomine exposure. Therefore, our study focused on the antibody response of guinea pigs experimentally exposed to saliva of different development stages of *T. infestans* from Argentina, Bolivia, Chile and Peru. Using IgG antibodies, 1D- and 2D-Western blotting analyses of guinea pig sera revealed a variety of detected salivary antigens from different *T. infestans* populations. IgG antibody responses demonstrated to remain detectable up to several months in animals exposed to triatomines. Thus, antigens recognized by IgG antibodies are not suitable for the detection of bugs at an early stage after insecticide sprayings of vector control programmes. Hence, IgM antibody responses of experimental guinea pigs were analyzed by 1D- and 2D- Western blotting. Despite the variability of IgM recognized antigens a potential exposure marker of 35 kDa was identified using both IgG and IgM antibodies of sera from laboratory experiments and from guinea pigs living in endemic Chagas disease areas of Bolivia. The specificity of this antigen was verified in cross-reactivity experiments, namely if antibodies elicited in guinea pigs by salivary proteins from different hematophagous arthropods cross-reacted in immunoassays with the candidate exposure marker. Sera of guinea pigs exposed to other triatomine species were also used to evaluate if the exposure marker is *T. infestans* specific. Additionally, peptides of a pallidipin-like protein identified as potential exposure marker from our previous study were synthesized and analyzed for their reactivity with guinea pig sera in immunoassays.

307

GENETIC ORIGIN OF HISTIDINE-RICH PROTEIN 2 GENE DELETION IN *PLASMODIUM FALCIPARUM* PARASITES FROM PERU

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A majority of commercially available malaria rapid diagnostic tests (RDTs) detect *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2), which is encoded by *pfhrp2*, a gene located subtelomerically on chromosome 8. Recently, it was determined that approximately 40% of *P. falciparum* isolates from the Peruvian Amazon lacked *pfhrp2*, leading to false-negative RDT results. We hypothesized that *pfhrp2*-deleted parasites in Peru may have derived from a single genetic event and expanded. To test this hypothesis, we examined historical samples collected between 1999 and 2005 to evaluate the haplotype structure of *pfhrp2* using microsatellite markers flanking the gene. Our data shows evidence for genetic deletions in at least four distinct *pfhrp2* haplotypes corresponding to four different clonal lineages of parasite populations collected in 1999-2001. Furthermore, there is evidence for recombination of these *pfhrp2* haplotypes in subsequent years (2003-2005) resulting in the emergence of hybrid haplotypes. These findings indicate that the genetic origin of *pfhrp2* deletion was not a rare event, as we had hypothesized, but may have occurred multiple times independently in parasites of different genetic backgrounds, partially explaining the widespread presence of *pfhrp2*-deleted parasites in Peru. Future investigations are necessary to determine the selective force(s), if any, acting on these population(s) that favor expansion of *pfhrp2* negative parasites.

308

QUANTITATIVE DETECTION OF *PLASMODIUM FALCIPARUM* HISTIDINE RICH PROTEIN 2 IN SALIVA OF CHILDREN WITH MALARIA

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Malaria is a global health priority with a heavy burden of fatality and morbidity. Improvements in field diagnostics are needed to support the agenda for malaria elimination. Saliva has shown significant potential for use in non-invasive diagnostics, but the development of off-the-shelf saliva diagnostic kits requires best practices for sample preparation and quantitative insight on the availability of biomarkers and the dynamics of immunoassay in saliva. This study measured the levels of the PfHRP2 in patient saliva. Matched samples of blood and saliva were collected between March and August, 2011 from forty patients at the ER and OPD of the pediatric unit of Korle Bu Teaching Hospital. Parasite density was determined from thick-film blood smears. Concentrations of PfHRP2 in saliva of malaria-positive patients were measured using a custom chemiluminescent ELISA in microtitre plates. Forty negative-control patients were enrolled. Saliva samples were stabilized with protease inhibitor.Of the forty patients with microscopically confirmed *Plasmodium falciparum* malaria, thirty seven tested positive for PfHRP2 in the blood using rapid diagnostic test kits, and forty for PfHRP2 in saliva. All negative-control samples tested negative for salivary Pf HRP2. The ELISA greed with microscopy with 100 % sensitivity and 100 % specificity. Salivary levels of PfHRP2 ranged from 15 to 1,162 pg/mL in the malaria-positive group. Saliva is a promising diagnostic fluid for malaria when protein degradation and matrix effects are mitigated. Systematic quantitation of other malaria biomarkers in saliva would identify those with the best clinical relevance and suitability for off-the-shelf diagnostic kits.

309

ANALYSIS OF THE PFHRP2 GENETIC DIVERSITY IN THREE AFRICAN COUNTRIES AND IMPLICATIONS FOR RAPID DIAGNOSTIC TEST EFFICACY

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The Senegalese National Malaria Control Program has recommended the use of Rapid Diagnostic Tests (RDTs) that target the Histidine Rich Protein 2 (HRP2), specific to *Plasmodium falciparum*, to diagnose malaria cases. The target antigen has been shown to be polymorphic which may explain the variability in HRP2-based RDT results reported in field studies. The genetic diversity of the pfhrp2 gene has not been investigated in depth in many African countries, including Senegal. The goal of this study is to determine the extent of polymorphism in pfhrp2 in three unique African populations: Senegal, Mali, and Uganda, and discuss the implications of these findings on the utility of RDTs based upon HRP2 detection. Sequencing data from the pfhrp2 locus was used to analyze the genetic diversity of this gene among three African populations Senegal, Mali, and Uganda with divergent transmission dynamics and malaria parasite ecology. Both single nucleotide polymorphisms (SNPs) in the pfhrp2 gene and amino acid repeat polymorphism were characterized, and parameters of genetic diversity in these populations were assessed. We observed extensive repeat length polymorphism in PfHRP2 antigen, and 67 mutations in the pfhrp2 gene including: Synonymous and Non-Synonymous single nucleotide polymorphisms (SNPs), insertions and deletions (INDELS), frame shifts, were identified; however, little diversity was observed at the nucleotide level (π=0.0000, π=0.00233, π=0.00289 for Senegal, Mali and Uganda respectively). Similar patterns were observed in the number, organization and the type of predicted amino acid repeats in the protein among the three populations, characterized by an occurrence of the Type 2, Type 4 and Type 7 repeats in the all populations studied. Type 4(AHH) and Type 7 (AHHAAD) are significantly different between the 3 populations with Pvalue=0.0067 and P=0.0287 respectively. The frequency and distribution of amino-acids repeats shows inter and intra-geographic variation in the PfHRP2 protein. The results provide insight about the genetic diversity of the pfhrp2 gene; and, based on the low genetic diversity observed, pfhrp2 seems to be a good candidate for RDTs design. However, since deletions of this gene has been observed in some endemic countries, it is crucial to focus on other essential genes as a targets for diagnostic tools.

310

DETECTION OF *PLASMODIUM* SPECIES CAUSING MALARIA IN LILONGWE, MALAWI

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Malaria RDT have played a major role in malaria management; particularly in providing blood based diagnosis in remote locations where microscopy is unavailable. These tests are fast and easy to perform and do not require electricity or specific equipment. As part of strengthening malaria diagnostics in Malawi, the Ministry of Health recommends use of RDTs in health care delivery system. However, microscopy remains a gold standard test for malaria. All patients with suspected uncomplicated malaria should have a confirmed diagnosis with RDT before antimalarial treatment is administered. Based on field performance evaluations that assessed performance, quality control and production capacities of the manufacturing companies of malaria RDTs, the Ministry of Health recommended two brands of Histidine Rich Protein 2 (HRP-2), RDTs for use in Malawi. These are SD Bioline malaria Ag *Pf* and the New Paracheck malaria Ag *Pf.* All these RDTs are able to detect only *Plasmodium falciparum.* However, other species have been reported to exist in the

country and there is need to find proper RDTs that will be able to detect and other species including *P. falciparum*. This study recruited a total of 250 adult and infants in Lilongwe, Malawi. The samples were processed at Bwaila Hospital Laboratory, UNC Project Laboratory and the University of North Carolina in Chapel Hill. Study results showed that the overall sensitivity and specificity of the Paramax-3 RDT used in the study were 100% and 83% respectively. However, it was observed that the RDT test was not able to identify the *P. ovale*, and in some cases, the RDT test was positive for *P. falciparum* when the PCR identified the species as *P. ovale*. No *P. vivax* was detected both by RDT and PCR. This study was able to detect and identify the presence of *P. malariae* and *P. ovale* in Malawi apart from the *P. falciparum*. There were no significant differences in between microscopy results compared to both the RDT and the PCR, with 94% and 98% sensitivities of R1 and R2 compared to RDT, as well as 94% and 96% sensitivities for R1 and R2 compared to PCR respectively. Both R1 and R2 had low specificities for example, R1 had 72% and R2 had 80% compared to RDT. Comparing R1 and R2 to PCR, the sensitivities were 64.9% and 67.2% respectively. However, the readers had difficulties differentiating the different species microscopically. The history of antimalarial treatment had no significant effect in the outcome of the results in both the RDT and PCR.

311

UTILIZATION OF MALARIA DIAGNOSTIC TESTS AND RECEIPT OF ANTIMALARIAL DRUGS BY FEBRILE PATIENTS ATTENDING OUTPATIENT DEPARTMENTS OF HEALTH CENTER IVS IN MUKONO DISTRICT, UGANDA

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Malaria remains a major public health problem in Uganda with annual estimates of 10 million cases and 43,000 deaths. Demonstration of the presence of malaria parasites prior to treatment with anti-malarial drugs is paramount to effective malaria case management. The aim of this study was to describe patients' factors associated with utilization of malaria diagnostic tests among febrile patients suspected of malaria and to help understand the role of utilization of malaria diagnostic tests in the receipt of anti-malarial drugs in a bid to better inform policy-makers on possible measures to minimize anti-malarial drug wastage at public health facilities. In a cross-sectional study design, client-exit interviews with febrile patients were conducted at health center IVs in Mukono district. Data were obtained with the aid of an interviewer-administered predetermined semi-structured questionnaire. Data entry and analysis were done using Epi-Data version 3.2 and STATA version 10.0 respectively. Frequencies and proportions were used to describe the sample population; chi square was used in two by two tables, odds ratios as the measure of association and an alpha level of 0.05 was used in all significance tests. Out of 472 potential participants screened for eligibility, 408 consented, almost half of whom were female (252, 61.8%) aged less than five years (120, 29.4%). There were no statistically significant differences between utilizers and non-utilizers in most characteristics except age, time of arrival at the health center, history of indoor residual spraying and overall satisfaction with services at out-patient-departments. Out of the 408 respondents, 359(88%) utilized malaria diagnostic tests and 241(59%) received anti-malarial drugs while 12(3%) neither utilized malaria diagnostic tests nor received anti-malarial drugs. In the adjusted analysis, utilizers were 75% less likely to receive anti-malarial drugs than non-utilizers after controlling for age, sex and type of place of residence (OR: 0.25, 95%CI: 0.09 , 0.66). Utilization of malaria diagnostic tests indeed has a bearing on the receipt of anti-malarial drugs. Efforts to minimize anti-malarial drug wastage should be therefore geared towards increasing utilization of malaria diagnostic tests.

312

IMPROVING DIAGNOSTIC CAPACITY FOR MALARIA AND MANAGEMENT OF FEBRILE ILLNESS IN UGANDA BY TRAINING LABORATORY HEALTH CARE WORKERS IN MALARIA DIAGNOSIS BY MICROSCOPY

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Malaria diagnosis with either microscopy or a rapid diagnostic test (RDT) is recommended for all cases of fever in Uganda to improve patient care, reduce unnecessary antimalarial use, improve surveillance, provide confirmation of treatment failures and allow for other diagnosis to be sought in negative cases. Coverage of malaria diagnostic services remains very low in Uganda and quality microscopy services are severely lacking. Since 2009, Uganda Malaria Surveillance Project (UMSP) in partnership with the National Malaria Control Program have trained laboratory personnel in malaria diagnosis. Training targeted all cadres of laboratory personnel from all health facilities in Uganda. The objective was to improve diagnosis capacity for malaria and management of febrile illness by training laboratory health care workers in malaria diagnosis by microscopy. The methods of Training consisted of a three-day course conducted at a centrally located district hospital or Health Centre IV with 75% of the time dedicated to practical sessions. Training impact was measured through a written examination and evaluation of the quality of blood slide preparation and accuracy of field microscopy. A total of 1462 laboratory personnel were trained from 21 districts. Average test scores improved from 41% to 75% ($p<0.001$). Sensitivity improved from 84% to 95% ($p<0.001$) and specificity improved from 87% to 97% ($p<0.001$). The proportion of well prepared blood smears improved from 6% to 75% ($p<0.001$). Therefore the refresher training significantly improved malaria diagnosis accuracy.

313

PRESENTING ATYPICAL LYMPHOCYTES AND *THROMBOCYTOPENIA* IN MALARIA INFECTION RESEMBLE TO DENGUE INFECTION

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Uncomplicated *Plasmodium falciparum* and *P. vivax* malaria patients usually present with acute febrile illness, atypical lymphocytosis and thrombocytopenia similar to dengue infection. To investigate the similarity manifestation, we retrospectively studied atypical lymphocytes (AL), atypical lymphocytosis (ALO) and platelet count in 1,310 uncomplicated malaria patients who admitted in the Bangkok Hospital for Tropical Diseases. 718 *P. falciparum* and 592 *P. vivax* malaria patients were enrolled into the study. In *P. falciparum* malaria patients, upon admission, AL and ALO were found in 53.2% and 5.7% of the patient, respectively (range 0-10%). While in *P. vivax* malaria patients, AL and ALO were seen in 55.4% and 9.5% of the patients, respectively (range 0-14%). After receiving antimalarial therapy, AL and ALO declined in both groups. However, AL and ALO remained occurred until the 4th week after admission. Interestingly, we also found that all patients presented with thrombocytopenia on admission. Although initial platelet counts were significantly lower than normal in both study groups, they slowly increased significantly over time and approached normal levels by days 28 post-treatment. In conclusion, AL or ALO as well as low platelet counts may be found in uncomplicated *falciparum* and *vivax* malaria mimicking dengue infection. In tropical countries where both dengue and malaria are endemic, presence of AL or ALO in any acute febrile patients with thrombocytopenia (similar to the findings in dengue) malaria could not be excluded. Particularly if the patients have risk of malaria infection, confirmative microscopic examination for malaria should be carried out.

314

ASSOCIATED FACTORS OF CIRCULATORY SHOCK IN ADULT PATIENTS WITH SEVERE *FALCIPARUM* MALARIA

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Infection with *Plasmodium falciparum* is a life-threatening medical condition particularly in severe malaria patients. Shock is a complication that frequently occurs and needs immediately clinical management. To investigate the risk factors for the development of shock in adult patients with severe *falciparum* malaria. We conducted a retrospective unmatched case-control study of the adult patients admitted to the Bangkok Hospital for Tropical Diseases, Thailand, between the years 2000-2010. One hundred patients with severe *falciparum* malaria and shock and 100 patients with severe malaria without shock were studied. Demographics, presenting signs and symptoms and initial laboratory data of those patients were analyzed. By statistical method, we found that 5 risk factors were identified as indicators of shock. These included female gender (OR 6.16, 95% C.I. 3.17 - 11.97), red cell distribution width (RDW) >15% (adjusted OR 2.90, 95% C.I. 1.11 - 7.57), anorexia (adjusted OR 2.76, 95% C.I. 1.03 - 7.39), hypoalbuminemia (adjusted OR 2.19, 95% C.I. 1.10 - 4.34), and BUN-Creatinine ratio >20 (adjusted OR 2.38, 95% C.I. 1.22 - 4.64). It is interesting that diarrhoea was found to be a protective factor (adjusted OR 0.33, 95% C.I. 0.14 - 0.78). We concluded that female gender, RDW >15%, anorexia, hypoalbuminemia, and BUN-Creatinine ratio >20 were risk factors of shock development in severe *P. falciparum* malaria patients. Further studies need to be compared with this data from different geographical areas, to construct practical measures to address potentially shock indicators in different settings.

315

PRESENTING *SCHIZONTEMIA* AND SEVERITY OUTCOME IN ADULT PATIENTS INFECTED WITH *PLASMODIUM FALCIPARUM* MALARIA

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Malaria, an infectious disease caused by protozoans of the genus *Plasmodium* continues to be a major health problem, particularly in the tropics and sub-tropics. Hyper*parasitemia* is a laboratory feature that commonly occurs in severe malaria patients. However, some malaria patients may undergo to severe malaria with initial low *parasitemia*. Schizontemia is a factor that may be caused of suffering in malaria patients. To investigate whether schizontemia associated with severe malaria condition and could be used as an indicator for predicting severe malaria. We retrospectively studied initial clinical and laboratory presentations upon admission in malaria patients admitted in the Bangkok Hospital for Tropical Disease. According to WHO criteria, 250 uncomplicated *falciparum* malaria cases and 250 severe *falciparum* malaria cases were enrolled in to the study. Based on statistical analysis method we found that presenting schizontemia were only detected in 99 severe malaria patients (39.6%). Moreover, presenting schizontemia also showed correlation with initial asexual parasite density. We concluded that presenting schizontemia in *falciparum* malaria might be helpful for clinicians in order to treat malaria patients since schizont stage was not found in uncomplicated *falciparum* malaria in this study. Further studies need to be carried out to compare with this data from different geographical settings, to construct a practical measure to address potentially presenting schizontemia in severe malaria patients in different settings.

316

MULTIPLEX QPCR FOR DETECTION AND ABSOLUTE QUANTIFICATION OF MALARIA

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We describe development of an absolute multiplex quantitative real-time PCR for detection of *Plasmodium spp.*, *P. falciparum* and *P. vivax* targets. Important qPCR experimental details and information that are important for the performance and reliability of PCR assay were investigated. Inhibition studies were performed to test and compare co-purification of PCR inhibitors in samples extracted from whole blood using either manual or automated methods. To establish the most optimal qPCR reaction volume, volume titration of the reaction master mix was performed starting at 10µL to 1µL of the reaction master mix with 1µL of template DNA in each assay reaction. As the reaction volume decreased, qPCR assays became more efficient with 1µL reaction master mix being the most efficient. For more accurate quantification of parasites in a sample, we developed plasmid DNAs for all the three assay targets for absolute quantification. All of absolute qPCR assays performed with efficiency of more than 94%, R^2 values greater than 0.99 and the STDEV of each replicate was <0.167. Linear regression plots generated from absolute qPCR assays were used to estimate the corresponding parasite density from relative qPCR in terms of parasite/µL. One copy of plasmid DNA was established to be equivalent to 0.1 parasite/µL for *Plasmodium spp.* assay, 0.281 parasites for *P. falciparum* assay and 0.127 parasite/µL for *P. vivax* assay. This study demonstrates for the first time use of plasmid DNA in absolute quantification of malaria parasite. The use of plasmid DNA standard in quantification of malaria parasite will be critical as efforts are underway to harmonize molecular assays used in diagnosis of malaria.

317

USE OF PET-PCR FOR THE MOLECULAR DETECTION OF MALARIA PARASITES IN HAITI NATIONAL MALARIA SURVEILLANCE STUDY

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We recently described a real-time PCR assay known as photo-induced electron transfer (PET)-PCR which relies on self-quenching primers for the detection of *Plasmodium spp.* and *P. falciparum*. PET-PCR assay is robust, less expensive and easier to use in resource limited countries when compared to currently available real-time PCR methods. Here, we investigated the potential of PET-PCR for molecular detection of malaria parasites in 2989 dried blood spots collected for national malaria Tracking Results Continuously (TRaC) community survey in Haiti, conducted in 2011. DNA from the dried blood spots was extracted using the QIAGEN method. All the 2989 samples were screened using the PET-PCR assay in duplicates. Samples with a cycle threshold (CT) of 40 or less were scored as positive. A randomly selected subset of the total samples (546) was tested using a nested PCR assay for confirmation. In addition, these same samples were also tested using a TaqMan-based PCR assay. A total of 12 out of the 2989 samples screened (0.4%) were found to be positive by PET-PCR. These same samples were also found to be positive by the nested and TaqMan-based methods. The nested PCR detected an additional positive sample that was not detected by either PET-PCR or TaqMan-based PCR method. The sensitivity and specificity of the PET-PCR compared to the nested PCR, as a gold standard, were found to be 92.3% (95% CI:

69.9%-100%) and 100% (95% CI: 99.1%-100%), respectively. Similar sensitivities and specificities were obtained for the TaqMan-based real-time PCR. PET-PCR yielded comparable results with other sensitive PCR methods and can be considered for rapid screening of large scale samples in surveillance studies.

318

DETECTION OF ANTIMALARIAL DRUG RESISTANCE: PERFORMANCE OF THE NOVEL HEMOZOIN SENSITIVITY ASSAY IN GABON

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Antimalarial-drug resistance is a threat for malaria control. Resistance has been described for many drugs, including the first-line treatment with artemisinins. *In vitro* antimalarial sensitivity testing is crucial to detect and monitor drug resistance. Currently available sensitivity assays have some drawbacks, including the detection of resistance in field isolates (e.g.: none detects artemisinin resistance). The recently developed hemozoin (Hz) detection assay showed to be able to overcome some of the limitations of existing assays. Promising results were obtained using *P. falciparum* continuous cultures in the laboratory. In this study we have determine the utility and performance of the Hz detection by directly culturing *ex vivo* blood samples from malaria Gabonese patients. Infected red blood cells were isolated and immediately incubated for 72-hours with increasing concentrations of chloroquine, artesunate and artemisinin. On site, a flow cytometer – Cyflow (Partec, Munster) was easily modified to detect Hz light depolarization. The percentage of Hz-containing infected red blood cells determined by flow cytometry was used as maturation indicator. Measurements were done at 24, 48 and 72 hours of incubation. Preliminary results showed that by assessing the percentage of Hz-containing infected red blood cells parasite maturation and antimalarial-drug inhibitory effects could already be detected after 24-hours of incubation. Inhibitory concentrations of 50% (IC50) were calculated at the same time-point. In the case of artemisinin compounds IC_{50} values were higher than the ones previously described using the Histidine-rich protein 2 (HRP2) assay. This might be a consequence of the assay's conditions, since it has been described that some drugs show an increased inhibitory concentration when greater numbers of parasites are inoculated. In the HRP2 assay parasite densities are adjusted to 2500 parasites/µl, while in the Hz detection assay they were not adjusted, thus analyzed samples had parasite densities ranging from 2400 parasites/µl up to 79200 parasites/µl. These results suggest that the rapid (24-hour), novel Hz assay could be used to determine the sensitivity/resistance pattern of the parasites in clinical isolates. This would be an useful tool not only in malaria endemic countries but also for rapid resistance testing in returning travellers.

319

PREVALENCE OF DRUG RESISTANCE ASSOCIATED MUTATIONS IN CLINICAL ISOLATES OF *PLASMODIUM VIVAX* FROM SOUTHERN PAKISTAN

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Antifolate antimalarial drugs sulphadoxine-pyrimethamine (SP) are the mainstay of malaria control in Pakistan. Though, not recommended, it is still being used largely in the public sector against *Plasmodium vivax*.Extensive use of SP indicates possibility of accumulation of drug resistance associated mutations in SP binding sites of *P. vivax*, encoded by *dihydrofolate reductase (dhfr)* and *dihydroptereoate synthethase* (*dhps*) genes. The aim of this study was to identify baseline frequencies of single nucleotide polymorphisms (SNPs) and prevalence of SP resistance associated mutations in clinical isolates of *P. vivax* (n=131) from Karachi, Sindh and Balochistan province. Nested PCR followed by direct sequencing and comparison with wild type reference sequences was performed. In *dhfr*, mutations were observed at codons F57L, S58R and S117N/T while novel non-synonymous mutations were observed at codon positions N50I, G114R and E119K .Two mutations, N50I and 117T were observed for the first time in Pakistan. The 50I mutation signifies intra species recombination of *P. falciparum* and *P. vivax* while 117T, isolated globally from treatment failure cases, shows the extent of SP pressure on *P. vivax*. In *dhps*, mutations were observed at codon position A383G and A553G while non-synonymous mutations were observed at codon positions S373T, E380K, P384L, N389T, V392D, T393P, D459A, M601I, A651D and A661V. Results from this study provide evidence that increasing number of SP resistance associated mutant alleles, comparable to those reported worldwide, are circulating in southern Pakistan. These alleles may play a significant role in transmission of resistant strains via human parasite reservoirs exacerbating extensive drug pressure on *P.vivax* and possibly making SP defunct for future use.

320

PLASMODIUM FALCIPARUM FIELD ISOLATES EX VIVO ANTIMALARIAL DRUG RESPONSE IN THIES (SENEGAL)

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Plasmodium falciparum malaria continues to be a major global cause of mortality and morbidity. Malaria treatment and control has been complicated by the emergence of resistance to widespread antimalarial drug use. Simple assays to monitor parasite drug response in clinical samples are important, as they can detect drug resistance before it becomes clinically apparent as well as inform changes in treatment policy to help prevent the spread of resistant parasites. We surveyed malaria cases in a clinic in Thies, Senegal from 2008-2012 using DAPI-based ex vivo drug assay to test *P. falciparum* response to amodiaquine, chloroquine, quinine, pyrimethamine, lumefantrine, piperaquine, mefloquine and artemisinin derivates in approximately 500 clinical isolates. Mutations in pfcrt and pfmdr1 were associated with changes in drug response, and we observed strong concordance between the ex vivo and *in vitro* IC_{50}s of culture adapted parasites. Thus surveillance of ex vivo drug sensitivity assays should be an integral part of the planned malaria control program so that resistance dynamics can be assessed and the most effective treatment can be selected or modified.

321

FIELD VALIDATION OF CANDIDATE MOLECULAR MARKERS OF ARTEMISININ RESISTANCE IN THAILAND

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The emergence of artemisinin resistance in Southeast Asia threatens the efficacy of artemisinin-based combination therapies (ACTs) and prospects of successful malaria elimination. Molecular markers of artemisinin resistance would provide useful tools for surveillance of resistance and help guide containment and elimination efforts. The first genome-wide association study of artemisinin resistance in clinical *Plasmodium falciparum* infections identified two candidate single nucleotide

polymorphisms (SNPs) on chromosomes 10 and 13 (MAL10-688956 and MAL13-1718319), that were significantly associated with delayed parasite clearance after treatment with oral artesunate. These SNPs were genotyped by pyrosequencing in 400 patient samples from eight sites in Thailand, collected between 2006 and 2011 as part of routine WHO Therapeutic Efficacy Surveys monitoring ACT treatment outcomes. Parasite clearance at these sites ranged from a half-life of 2 hours, corresponding to sensitive parasites, to up to 7 hours in some regions, comparable to slow-clearing artemisinin-resistant parasites described in western Cambodia. Prevalence of both SNPs varied greatly by region, ranging from less than 10% to fixation at 100% across sites. The association between the presence of each SNP on day 0 prior to treatment with the prevalence of *parasitemia* on day 3 and PCR-confirmed recrudescence after treatment was estimated as a means of validating these candidate molecular markers as predictors of clinical artemisinin resistance. Odds ratios for association between SNPs and day 3 *parasitemia* will be reported. This study represents the first attempt to validate these candidate molecular markers in an independent set of clinical samples from areas of Thailand with known artemisinin resistance.

322

ANALYSIS OF THERAPEUTIC EFFICACIES OF AMODIAQUINE-ARSTESUNATE AND ARTEMETHER-LUMEFANTRINE FOR TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN BURKINA FASO FIVE YEARS AFTER THEIR IMPLEMENTATION

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Since 2005, Burkina Faso adopted artesunate plus amodiaquine (ASAQ) and artemether-lumefantrine (AL) as first-line treatment for uncomplicated malaria. Despite improvement in that treatment, malaria remains the first cause of morbidity and mortality in the country. This study aimed to analyze the therapeutic efficacies of ASAQ and AL for the treatment of uncomplicated *falciparum* malaria in Burkina Faso five years after their adoption. Per-protocol individual data from four randomized clinical trials supported by IRSS-DRO Bobo Dioulasso in 2006, 2008, 2009 and 2010, including 1076 patients with uncomplicated *Plasmodium falciparum* malaria, treated with the recommended regimen of AL or ASAQ, were analyzed according to WWARN analytical methods. Patients benefited from a clinical and biological 28-day follow-up and performed on days 2, 3, 7, 14 and 28 to evaluate clinical and parasitological outcomes. Treatment failures have been corrected by PCR. Results: Using WWARN analytical methods, the unadjusted Kaplan-Meier survival estimates are 76.4% (95% CI (72.5-79.8)) in the AL group (N=544) and 87.1% (95% CI (83.9-89.7)) in the ASAQ group (N=532). After PCR correction, AL was less efficacious than ASAQ respectively 95.8% (95% CI (93.6-97.3)) vs 98.2% (95% CI (96.6-99.1)); OR=0,486 (95% CI (0.217-1.089). There was no significant correlation between the occurrence of recrudescent at day 28 end-point and study year in two groups (coefficient<0.1). Conclusion: AL and ASAQ remain effective as treatment for uncomplicated malaria according to WHO recommendations, though AL was inferior in preventing recrudescent for 28-day follow-up.

323

ASSESSMENT OF RESISTANCE MARKERS LEVEL FOR ARTESUNATE + AMODIAQUINE COMBINATION FOR THE TREATMENT OF THE UNCOMPLICATED MALARIA IN MAFERINYAH, GUINEA

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The use of the Amodiaquine in monotherapy, is associated with the selection of resistance markers (*pfcrt* K76T and *pfmdr1*86Y). Although there is not documented resistance, no resistance markers to artemisin derivatives it is important to assess the impact of artemisin based combination therapy (ACT) on the selection of markers associated with partner molecules. This study was undertaken **t**o evaluate the efficacy of Artesunate+Amodiaquine combinationin the treatment of uncomplicated malaria in Maferinyah, Guinea Conakry; - To search for punctual mutations on Amodiaquine resistance genes (*Pfcrt* 76T and *Pfmdr1*-86Y). - To assess for polymorphisms (MSP1, MSP2 and CA1) in order to discriminate new infection versus recrudescence. We assessed *invivo* efficacy of Artsunate+Amodiaquine (AS+AQ) on subjects aged from 3 months to 45 years living in Mafrinyah, near Conakry in Guinea. The efficacy of AS+AQ has been evaluatedby WHO 28 days standard *invivo* test. Polymorphisms (MSP1, MSP2 and CA1) and punctual mutations on Amodiaquine resistance genes (*Pfcrt*76T and *Pfmdr1*-86Y) have been determined by PCR. A total of 93 samples have been randomly selected before treatment and 11 samples with *parasitemia* after treatment have been analyzed. Baseline frequencies of *Pfcrt*76T and *Pfmdr1* mutations were respectively 67.7% (63/93) and 31.1% (28/93). These frequencies after treatment were respectively 50% for *Pfcrt*76T and 54% for *Pfmdr1* 86Y. In conclusion, these data show an increased baseline level of *Pfcrt*76T gene and a significant selection of AQ molecular maker trough AS+AQ.

324

IN VIVO EFFICACY AND MOLECULAR RESISTANCE MARKERS OF SULFADOXINE-PYRIMETHAMINE FOR INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY — MANSA, ZAMBIA, 2010

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Intermittent preventive treatment of malaria in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) reduces adverse effects of malaria infection. Emergence of SP-resistant *Plasmodium falciparum* threatens this strategy. The quintuple mutant haplotype, mutations in the *dhfr* and *dhps* genes, is associated with SP treatment failure in non-pregnant patients. Zambia has implemented its SP IPTp program since 2003. We sought to determine the efficacy of SP IPTp and the presence of the quintuple mutant, neither previously described among HIV-negative pregnant women in Zambia. In Mansa, Zambia, HIV-negative pregnant women presenting to antenatal clinic for the 1st dose of SP IPTp with asymptomatic *parasitemia* were enrolled and tested for *parasitemia* weekly for 7 weeks. Outcomes were parasitological failure (PF, *parasitemia* during follow-up), and adequate parasitological response (APR, no *parasitemia* during follow-

up). Polymerase chain reaction (PCR) distinguished recrudescence (failure) from reinfection, and identified molecular markers of SP resistance. Survival analysis was done; those who had incomplete follow-up (at least one follow-up day) or reinfection were censored. Of the 108 women enrolled, 51 (46%) completed the study, 34 (31%) had incomplete follow-up, 7 (6%) reinfection, and 17 (16%) were lost to follow-up after day 0 (LTFU). Of those who completed the study, PF occurred in 8 (16%), and APR in 43 (84%). For the 92 women included in survival analysis, median age was 20 years (range 15-39), median gestational age was 22 weeks (range16-28), and 57% were primigravid. There was no difference in time to failure in primigravid versus multigravid women. Of the 84 women with complete haplotype (includes those LTFU), 53 (63%) had quintuple or sextuple mutants. PF occurred in 22% of women with quintuple mutation versus 0% without quintuple mutation (p=0.44). While underpowered and possible bias due to high LTFU, this study shows low failure rates and incomplete penetration of the quintuple mutant. The threat of SP resistance looms, but SP IPTp may remain efficacious in Mansa.

325

MOLECULAR-BASED ANTIMALARIAL RESISTANCE MONITORING FOR *PLASMODIUM FALCIPARUM* IN NICARAGUA

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Plasmodium falciparum malaria is a potentially fatal disease; the regular monitoring of antimalarial efficacy is essential to inform national malaria policies. In Nicaragua, chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) are used as primary and secondary drugs, respectively, for treating uncomplicated *P. falciparum* malaria. Since *P. falciparum* incidence in Nicaragua is extremely low (236 cases in 2012), it is extremely difficult to conduct WHO-recommended *in vivo* efficacy trials to assess the efficacy of CQ and SP. In order to monitor antimalarial resistance, the Ministry of Health and its partners implemented a molecular-based antimalarial resistance surveillance system in 2010. Six sentinel sites in the Región Autónoma Atlántica Norte, where most *P. falciparum* malaria cases occur, were established in mid 2010. Following national guidelines, all *P. falciparum* malaria cases are admitted as inpatients for laboratory confirmation and treatment. A sample in filter paper is collected upon admission from all patients and, together with the malaria smear, is later sent to the national reference laboratory, Centro Nacional de Diagnostico y Referencia (CNDR), in Managua. CNDR staff confirms *P. falciparum* infection using microscopy and processes filter papers to evaluate for mutations commonly associated with CQ (*pfcrt*) and SP (*dhfr* and *dhps*) resistance by DNA sequencing. A total of 84 samples (28 in 2010 and 56 in 2011) were collected and sequenced for molecular markers. No resistance alleles known to be associated with CQ or SP resistance were detected. This finding also highlights how the use of molecular-based antimalarial resistance surveillance for both CQ and SP can provide valuable information to monitor for antimalarial resistance in countries such as Nicaragua, where malaria incidence has decreased to levels that impair the ability to conduct *in vivo* trials due to low number of patients eligible for enrollment.

326

PFDHFR AND PFDHPS GENE MUTATIONS ASSOCIATED TO CHEMORESISTANCE IN *PLASMODIUM FALCIPARUM* ISOLATES FROM LUBANGO, ANGOLA

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Malaria is a major parasitic disease. *Plasmodium falciparum* parasites are responsible for severe morbidity malaria cases and its chemoresistance is notorious. Thus, it becomes necessary to know the sensitivity of *P. falciparum* parasites to sulphadoxine / pyrimethanine (SP), to evaluate the effectiveness of pregnant women intermittent preventive treatment (IPT) used in Angola since 2006 as well as the appropriateness of IPT introduction in children under 5 years old, in this country. For this purpose, among the 107 blood samples collected from malaria patients in Lubango, Angola, and subjected to DNA sequencing we observed: 46% with double mutant haplotypes ACNRNVI in codons 59 and 108; 27% with double mutations in codons 51 and 108 showing the haplotype ACICNVI, 17% triple mutant at codons 51, 59 and 108 with haplotypes ACIRNVI; 2% triple mutant at codons 50, 51, 108 and 59, 108, and 164 with haplotypes ARICNVI ACNRNVL; 6% with a single mutation at codon 108, showing ACNCNVI haplotype and; only one wild isolate - ACNCSVI (2%). The 108N mutation (change of serine for asparagine - S108N) in *pfdhfr* gene - a mutation described as the predecessor for all resistant emergence strains - was the most prevalent (98%) followed by 59R (65%) and 51I (47%) mutations. Regarding *pfdhps* gene, a total prevalence of 437G mutation, with the exception of one sample (4%) who presented wild haplotype SAKAA were noted. Among the mutant samples, 88% had a single mutation presenting haplotype SGKAA; 6% showed double mutants at codons 437 and 540 originating haplotype SGEAA and; 6% displayed also a double mutant but at codons 436 and 437, with haplotype AGKAA. Quadruple (triple *pfdhfr* + single *pfdhps*) and triple mutants (double *pfdhfr* + single *pfdhps*) were recorded. Considering the significant percentage of *P. falciparum* parasites circulating in Lubango with a resistance profile associated with pyrimethamine and a tendency to sulphadoxine tolerance, we conclude that the IPT should not be effective in endemic areas of Angola.

327

DECREASED EX VIVO SENSITIVITY TO ARTEMISININ AND AMODIAQUINE AMONG *PLASMODIUM FALCIPARUM* PARASITES IN THIÉS, SENEGAL

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Plasmodium falciparum malaria continues to be a major global cause of mortality and morbidity. Malaria treatment and control has been complicated by the emergence of resistance to widespread antimalarial drug use. Simple assays to monitor parasite drug response in clinical samples are important, as they can detect drug resistance before it becomes clinically apparent as well as inform changes in treatment policy to help prevent the spread of resistant parasites. We tested parasite drug responses and genotyped drug resistance-associated mutations in approximately 400 *P. falciparum* malaria infections in Thiés, Senegal between 2008 and 2011. Over this time period, parasites became increasingly more resistant to both amodaquine and artemisinin, two

compounds deployed in artemisinin combination therapies (ACTs) in Senegal beginning in 2006. Additionally, the prevalence of several known resistance-associated mutations in *pfcrt* and *pfmdr1* also increased between 2008 and 2011. Increased amodiaquine resistance was associated with sustained, highly prevalent mutations in *pfcrt*, and one mutation in *pfmdr1* - Y184F - was associated with lowered parasite response to artemisinin. These data support the hypothesis that the use of amodiaquine and artemisinin derivatives in combination therapies is selecting for increased drug tolerance in this population. Thus surveillance using *ex vivo* drug sensitivity assays should be an integral part of the planned malaria control program, so that resistance dynamics can be assessed and the most effective treatment can be selected or modified.

328

A REPLICATION GENOME-WIDE ASSOCIATION STUDY OF THE GENETIC BASIS OF DELAYED PARASITE CLEARANCE FOLLOWING TREATMENT WITH ARTEMISININS

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Ongoing efforts to contain artemisinin-resistant malaria are hampered by the lack of tools to gauge the extent and direction of its spread. A molecular assay to detect markers of artemisinin resistance would be a highly valuable surveillance tool, but the genetic basis of resistance is unknown. An initial genome-wide screen with a relatively sparse set of markers identified single nucleotide polymorphisms (SNPs) on multiple chromosomes that were associated with delayed parasite clearance, as well as several regions of the parasite genome under recent positive selection. However, replication studies and denser coverage of the genome will be required to more finely map the location of genes involved in artemisinin resistance. In this study, we will replicate the genome-wide association study in an independent set of samples collected during artesunate efficacy studies conducted in Cambodia, Laos, Vietnam, and Myanmar. *P. falciparum* SNPs will either be genotyped using a high-density Nimblegen DNA microarray or called from short-read sequencing data. Linear mixed models and Random Forests will be used to estimate associations between individual SNPs and parasite clearance half-life, while adjusting for important covariates and taking into account multiple comparisons. Candidate markers of artemisinin resistance identified within high-priority genes located in validated genomic regions will be presented.

329

PREDICTORS OF SEVERE MALARIA IN CHILDREN UNDER FIVE YEARS OF AGE IN BURKINA FASO

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In endemic and sub-Saharan countries with high incidence and death of malaria in children, malaria eradication has been a key objective for many states. Malaria eradication program is a major public health priority in Africa, but finding an effective strategy of fighting is a challenging task. In Burkina Faso, most hospitals face the curse of malaria without access to pediatric intensive cares. Understanding the various factors which contribute to the severity of malaria is very difficult and there is a little information. To assess predictors of severe malaria for children, we assume that the incidence of clinical form of severe malaria is unknown and there are unknown predictors of severe malaria with its consequencies for contributing to increase the mortality. A total of 510 children (mean age 23.5 months) with suspect malaria were drawn from one District and one Regional Hospital of Koudougou from July to September 2012 using the cross sectional study. Each child was screened using blood smear to identify whether he/she had severe malaria using the criteria set by the World Health Organization. When a child is identify as having malaria, either severe or no severe malaria, the main caregiver is interviewed by a trained interviewer using a structure questionnaire. To describe the association between factors and malaria, logistic regression approach was utilized using SPSS 17.0. Out of 510 children, 53,3% were blood smear positive, 35.49% were not able to determine and 11.28% were negative but showed a clinical signs of malaria. 201 (39.4%) were severe malaria. Most of the patients (54.9%) were living in rural area. Of 29 (14.4%) who died with severe malaria, 16.1% died from anemia. Major predictors for severe malaria and its deaths were: rural area (11.6; p=.001); low income (OR=9.6;p=.001); illiteracy (OR=14.8; p=.001); cost of treatment (OR=10.9; p=.001); low hemoglobin (OR=496, p=.001); Hyper*parasitemia* (OR=23.5; p=.001), travel time (OR=4.1; p=.001); and self treatment (OR=2.7; p=.002). These findings are strongly consistent with previous studies in Malawie, Senegal, Cameroon, Zambia and Burkina Faso respectively. We found that severe malaria is still a serious public health concern for young children in resources limited setting. There is need for more health education, reducing cost of treatment, improve socio economic status, improve access to health facilities, and encourage early care seeking.

330

MALARIA DIAGNOSTIC SERVICES AND TREATMENT PRACTICES FOR FEBRILE CHILDREN UNDER 5 YEARS - MAKARFI, NIGERIA

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Malaria is the leading cause of childhood mortality in Nigeria. Averagely, children <5years (U5) are prone to three episodes annually. In 2005, the national malaria policy recommended Artemisinin-based combination therapy (ACT) due to established resistance to Chloroquine (CQ). In 2011, the policy was revised to ensure parasite-based diagnosis before treatment of malaria. However, treatment remains largely presumptive. We conducted a hospital-based cross-sectional study in a low malaria prevalence setting to determine awareness of malaria diagnostic services (MDS) and treatment practices for fever among caregivers of febrile U5 (FU5). We interviewed consecutively selected caregivers of 295 FU5, attending Makarfi General Hospital, Kaduna state, Nigeria; from December 2010 to August 2011. We included all eligible FU5 without rash. Information on factors influencing awareness of MLS and pre-hospital treatment (PHT) was collected. We examined the Giemsa-stained blood smear of FU5 for malaria. Fifteen (5.1%) caregivers have ever heard about MDS. Eleven (3.7%) caregivers were ever offered MDS by physicians. Being formally educated (Prevalence Odds ratio (POR): 0.05, 95% Confidence

Interval (CI): 0.01-0.20), living <5km from a health facility (POR: 4.21, CI: 1.39- 12.55), being a government staff (POR: 9.18, CI: 1.74- 39.93) and ever being offered MDS (POR: 35.09, CI 10.13-134.00) were positively associated with awareness of MDS. Overall, 201(67.9%) children had received any PHT, 121 children (41.0%) at patent medicine stores. Of the 31(10.5%) FU5 diagnosed with malaria and 264 (89.5%) without malaria diagnosis, 13 (41.9%) and 65 (24.6%) had PHT with CQ respectively. Awareness of MDS remains low. Treatment of FU5 against malaria is predominantly inappropriate. There is a need to sensitise caregivers and health staff on use of ACTs and adherence to confirmatory malaria diagnosis.

331

COMBINING DATA FROM MULTIPLE SPATIALLY REFERENCED SURVEYS: GEOSTATISTICAL ANALYSIS OF CHILDHOOD MALARIA IN CHIKHWAWA DISTRICT, MALAWI

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Geostatistical methods are becoming more widely used in epidemiology to analyze spatial variation in disease prevalence. These methods are especially useful In resource-poor settings where disease registries are either non-existent or geographically incomplete, and data on prevalence must be obtained by survey sampling of the population of interest. In order to obtain good geographical coverage of the population, it is often necessary also to combine information from multiple prevalence surveys in order to estimate model parameters and for prevalence mapping. However, simply fitting a single model to the combined data from multiple surveys is inadvisable without testing the implicit assumption that both the underlying process and its realization are common to all of the surveys. We have developed two classes of bivariate generalized linear geostatistical models (GLGM's) to combine data from two spatially referenced surveys so as to address each of two common sources of variation across surveys: variation in prevalence over time; and variation in data-quality. In the case of surveys conducted at different times, we assume an autoregressive dependence between the two components of the bivariate process. In the case of surveys that differ in quality, we assume that one of the surveys provides a gold standard whilst the other is potentially biased. For example, one survey might use a random sampling design, the other an opportunistic convenience sample. For parameter estimation in either models, we have developed a rapid Monte Carlo method for approximate evaluation of the likelihood function. Both approaches can easily be extended to analyze data from more than two surveys. We describe an application to malaria prevalence data from Chikhwawa District, Malawi. The data consist of two Malaria Indicator Surveys (MIS's) and an Easy Access Group (EAG) study, conducted over the period 2010-2012. In the two MIS's, the data were collected by random selection of households in an area of 50 villages within 400 square kilometers, whilst the EAG study enrolled a random selection of children attending the vaccination clinic in Chikhwawa District Hospital. The second sampling strategy is the more economical, but the sampling bias inherent to such "convenience" samples needs to be taken into account.

332

ETHIOPIA, NATIONAL MALARIA INDICATOR SURVEY 2011 (MIS-2011): COVERAGE AND USE OF MAJOR MALARIA CONTROL INTERVENTIONS

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Mmalaria still remains major public health problem in Ethiopia. Malaria Indicator Survey (MIS) had been conducted in 2007 aimed at measuring key malaria interventions coverage and prevalence of malaria morbidity as well as *parasitemia* and under five children anemia. MIS-2011 has been conducted from October to December 2011 to measure the progress of malaria prevention and control efforts undertaken since 2007 and see whether the goals set forth in the FMOH National Strategic Plan for Malaria Prevention and Control 2005 - 2010 were achieved. The survey was a national level study that used a two-stage random cluster sample of 10,444 households in 440 census enumeration areas. A total of 47,248 people participated in the survey. Data were collected using Roll Back Malaria M&E Reference Group household and women's questionnaires, which were adapted to the local context and assisted by PDA. Data collected were transferred in MS access data base and analysed using STATA and SAS statistical soft-wares. The results indicated that 55.2% of households have at least one mosquito net (of any type), and 54.8% of households have at least one long-lasting insecticidal net (LLIN). Of children U5, 38.2% slept under a net the night before the survey, and 64.5% of children U5 slept under a net in a household that owned at least one net. These figures were 35.3% and 63.8% respectively for pregnant women. IRS had been conducted in 46.6% of households in the last 12 months preceding the survey. It was reported that 19.7% of children U5 had suffered from a fever in the two weeks preceding the survey. Of these children, 51.3% sought medical attention within 24 hours of onset of fever; 32.6% took an antimalarial drug and 8.5% took the drug on the day of fever onset. Among the febrile children who were treated with an antimalarial on the day of fever onset, 68.9% sought their treatment from public health facilities. Malaria parasite prevalence in areas <2,000m was 1.3% by microscopy blood-slide examination for all ages, with 1% of these being *Plasmodium falciparum* and 0.3% being *P. vivax.* Compared to the MIS 2007 result, it is observed that net ownership use decline from 65% to 55.8%. However, IRS coverage grew from 20 to 46.6 % . Except some indicators, the findings of the survey show, all in all, the malaria control program in Ethiopia has sustained the gains in malaria control and prevention and the results implicate the right direction of the country in achieving its target.

333

THE EFFECT OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY ON THE INCIDENCE OF MALARIA AND OTHER DISEASES IN CHILDREN LIVING ON THE COAST OF KENYA

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It is widely believed that the prevalence of glucose-6-phosphate dehydrogenase deficiency (G6PDd) in human populations reflects selection by malaria. Nevertheless, few detailed epidemiological studies have investigated the impact of G6PDd on the risk of either malaria or of other common diseases among children living in malaria-endemic areas. Studies that have been conducted have yielded confusing results, one potential explanation being a lack of uniformity regarding the methods used to define G6PDd. We have investigated the impact of G6PDd both on the risk of malaria and other diseases among children living in Kilifi District on the coast of Kenya, where previous studies have shown that the only significant cause of G6PDd is the G6PD A- variant. In a study involving 1332 case-children presenting to Kilifi District Hospital with severe *Plasmodium falciparum* malaria and 7500 controls recruited from within the same geographic area we found that heterozygous females with the G6PD A- allele were significantly protected against severe *P. falciparum* malaria (OR 0.75; 0.61-0.91; p=0.004). Protection was also seen among homozygous females, although significance was lost on adjusting for confounding factors (0.70; 0.39-1.23; p=0.22). No protection was seen among hemizygous males (OR 1.06; 0.43-1.11; p=0.135) who, to the contrary, showed an increased risk of severe malaria anaemia (1.80; 1.24-2.61; p=0.002). In a cohort study conducted in the same geographic

area we found no effect of the G6PD A- allele on the incidence of either uncomplicated *P. falciparum* malaria or that of any other common childhood diseases with the exception of undiagnosed febrile illnesses, which occurred significantly less frequently among heterozygous females (IRR 0.61; 0.41-0.92; p=0.02). *P. falciparum* parasite densities were lower in G6PD A- carriers than normal children during episodes of clinical malaria but this only reached significance among homozygous females with uncomplicated disease. Through a range of studies conducted in an area where the G6PD A- variant is the only major cause of G6PDd we find that protection from severe malaria is limited to heterozygous females while hemizygous males are significantly predisposed to severe malaria anaemia. Reduced parasite densities in G6PD A- carriers are consistent with a mechanism involving enhanced clearance of *P. falciparum* -infected G6PDd red blood cells.

334

INCREASING INCIDENCE OF *PLASMODIUM KNOWLESI* MALARIA FOLLOWING CONTROL OF *P. FALCIPARUM* AND *P. VIVAX* MALARIA IN SABAH, MALAYSIA

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The simian parasite *Plasmodium knowlesi* is a common cause of human malaria in Malaysian Borneo and threatens the prospect of malaria elimination. However, little is known about the emergence of *P. knowlesi*, particularly in Sabah. We reviewed Sabah Department of Health records to investigate the trend of each malaria species over time. Reporting of microscopy-diagnosed malaria cases in Sabah is mandatory. We reviewed all available Department of Health malaria notification records from 1992-2012. Notifications of *P. malariae* and *P. knowlesi* were considered as a single group due to microscopic near-identity. From 1992-2012 total malaria notifications decreased dramatically, with *P. falciparum* peaking at 33,153 in 1994 and decreasing 46-fold to 716 in 2012, and *P. vivax* peaking at 15,857 in 1995 and decreasing 33-fold to 478 in 2012. Notifications of *P. malariae /P. knowlesi* also demonstrated a peak in the mid-1990s (614 in 1994) before decreasing to ≈100/year in the late 1990s/early 2000s. However, *P. malariae /P. knowlesi* notifications increased >10-fold between 2004 (n=59) and 2012 (n=815). In 1992 *P. falciparum* , *P. vivax* and *P. malariae /P. knowlesi* monoinfections accounted for 70%, 24% and 1% respectively of malaria notifications, compared to 36%, 24% and 40% in 2012. The increase in *P. malariae /P. knowlesi* notifications occurred state-wide, appearing to have begun in the southwest and progressed north-easterly. We conclude that a significant recent increase has occurred in *P. knowlesi* notifications following reduced transmission of the human *Plasmodium* species, and this trend threatens malaria elimination. Determination of transmission dynamics and risk factors for *knowlesi* malaria is required to guide measures to control this rising incidence.

335

DATA AND MODELS TO QUANTIFYING THE ROLE OF HUMAN TRAVEL IN MALARIA EPIDEMIOLOGY

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Human movements contribute to the transmission of malaria worldwide. Identifying sources and sinks of imported infections due to human travel and locating high-risk sites of parasite importation could greatly improve public health control programs. Here, we use spatially explicit mobile phone data and simulated disease models to identify the dynamics of human carriers of pathogens between regions. We address a number of issues with modeling human travel for malaria control and available data sources.

336

A DEEPER INSIGHT INTO IDENTIFYING MALARIA HOTSPOTS: TOPOGRAPHY, CLIMATE AND GENOTYPE STRUCTURE

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A microgeographic scale study is conducted in a 200×150 km2 area of western Kenya to investigate the impacts of environmental, spatial, and genetic factors on malaria prevalence. Approximately 13,000 samples collected in 2011-2012 representing 47 sites in western Kenya were examined. PCR- and microscopy-based measures indicate a strong and inverse correlation between elevation and prevalence rate. In addition, temperature, evapotranspiration rate, and wind speed are significantly related to prevalence. When all variables are included in the principal component analyses, we found that areas of relatively lower prevalence (<5%) occupy the greatest range of environmental amplitude compared to areas of higher prevalence (>25%). This suggests that topography and climate are generally more homogeneous in areas where malaria is prevalent and endemic. Geostatistic analyses using prevalence data indicate that the number and position of sampling points influence both spatial structure of prevalence and risk predictability within the studied area. The number of predicted malaria hotspot decreases when sampling points are reduced systematically in each of the iterations. This demonstrates that the detectability and precision of malaria hotspots are highly dependent of the number of sites being examined. Further, we utilize Fluidigm technology to obtain SNP genotypes of malaria parasite across the studied sites. A total of 72 SNPs representing both synonymous and non-synonymous changes in the exons and introns of various genes, and intergenic regions of the *Plasmodium falciparum* genome were assessed by the Fluidigm EP1 system and dynamic arrays. These SNPs are shown to provide a genetic signature of individuals that allow us to identify polyclonal samples as well as to scrutinize genotypic distribution among populations. Findings of environmental, spatial, and genetic analyses altogether shed light on factors and patterns of malaria transmission.

337

RELATIVE CONTRIBUTION OF *BACTEREMIA* AND MALARIA TO UNEXPLAINED ACUTE UNEXPLAINED FEVER IN UNDER-FIVE CHILDREN IN RURAL WESTERN KENYA

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Malaria and *bacteremia* are common causes of under-five febrile illness and mortality in sub-Saharan Africa. The two conditions are clinically indistinguishable and, while rapid diagnostic tests for malaria are now widely available in many settings, microbiologic laboratories to diagnose

bacteremia are very limited. Therefore studies of factors that can inform differential diagnosis of malaria and *bacteremia* in resource poor settings are critical to the appropriate management of febrile children and rational use of antimalarials and antibiotics. We determined the relative prevalence and predictors of malaria and *bacteremia* among febrile (axillary temperature ≥ 37.5°C) children consecutively presenting at two regional rural hospitals in Western Kenya. We collected detailed demographic, anthropometric, medical and clinical examination information from consenting children aged 6-59 months. Malaria positivity was determined by smear microscopy or rapid test. We performed blood cultures, isolate identification and antibiotic susceptibility testing using BACTEC™ 9050 and MicroScan Walkaway40® systems. All children were also tested for HIV. We studied 546 children of which 91 (16.5%) had malaria and, 20 (3.7%) had clinically significant *bacteremia*. Only 6 (1.1%) children were co-infected with malaria and *bacteremia*. Among children with *bacteremia*, *salmonella* strains were the most common isolates identified (12/20, [60%]). Factors significantly associated with malaria in multivariate analysis included being >2y old (OR=2.72; 95% CI: 1.45-5.08), presence of ≥1 WHO-integrated management of childhood illness (IMCI) danger signs (OR=4.11; 95% CI: 2.28-7.41), and consumption of unsafe water (OR=2.52; 95% CI: 1.26-5.03). We found no association between *bacteremia* and risk factors such as HIV, malnutrition, use of unsafe water, or presence of a WHO-IMCI danger sign. Unlike *bacteremia*, malaria is common among febrile children in rural Western Kenya, especially among those older than 2 years, manifesting IMCI danger signs, and consuming untreated water.

338

THE MIGRATORY PATTERNS OF *PLASMODIUM FALCIPARUM* MALARIA PARASITES BETWEEN ISLANDS OF THE LAKE VICTORIA REGION, KENYA

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Malaria is responsible for extensive mortality and morbidity with the current statistics recording approximately 660,000 deaths and 220 million malaria cases globally in 2010.Today, 11 countries in Africa have embarked on malaria elimination. Although the continent is witnessing an epidemiological transition with plummeting malaria risks, the feasibility of malaria elimination in settings of high transmission tropical Africa remains unclear.A molecular epidemiological survey aimed at generating baseline data in a malaria endemic site to reveal patterns of disease transmission across the Lake Victoria islands is currently ongoing. The contribution of inter-island human, parasite and mosquito migration to the maintenance of malaria transmission will be investigated for the effective planning of malaria interventions. Following the application of malaria control measures, the likelihood of malaria reappearing due to reintroduction of parasites from outside the control region can be assessed. In addition, reductions in malaria endemicity may lead to the emergence of clusters or foci of malaria transmission. These can also be identified and control measures scaled up for effective and sustained malaria elimination. *Plasmodium falciparum* isolates were collected from various locations on the islands and the surrounding mainland, and parasites DNA extracted. The samples were then genotyped using eight genome-wide microsatellite markers. Population genetics analyses were then performed in order to investigate the population dynamics of parasites from the study area with emphasis on determining whether there are distinct foci of transmission, or whether the population is in panmixia. . Further genotyping was performed on *P. falciparum* merozoite surface proteins 1 and 2 (Pfmsp1 and Pfmsp2) to evaluate the degree of multiplicity of infection in each region. We will present the results of this parasite population profiling, and discuss the implications of our findings for the efforts to eliminate malaria from this region.

339

TREND OF MALARIA MORBIDITY IN KERSA, SOUTHWEST ETHIOPIA

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Malaria is a highly infectious disease, causing the major cause of disease and death in sub-Saharan Africa, especially among children and pregnant women. This paper assessed the trend of malaria cases from September 2005 to August 2011. A retrospective analysis of daily outpatient consultation records was obtained from Jimma Health Bureau. Moreover, One year cross-sectional blood film examination was performed in Bulbul, Serbo and Bala Wajo health centers in Southwest Ethiopia.Data were entered and checked, thereafter analyses were performed using excel and SPSS version 16 software. Descriptive statistics was used to assess the trend of malaria cases detected over six year period. We assessed 6 years trend of malaria case in Kersa area between September 2005 to August 2011. A total of 57482 malaria cases were diagnosed in the three Health centers. Among these, a total of 15865 were children under five years of age. The majority (88.80%) of malaria cases were reported in 2006/2007. Moreover, the percentage of *Plasmodium falciparum* and *P. vivax* were *57.37%* and 42.63% respectively. To minimize reliability and validity of secondary data, one year cross sectional analysis was performed in Kersa Woreda from three health centers. Concerning the one year cross-sectional study, males were more affected (60.1%) than females (39.9%). In the same year 33.8% of the positive cases were children. The proportion of malaria cases detected among clinical suspects over the 5 year period was 51.13% .On the other hand the proportion of malaria cases was during one year blood film examination was 25.32%. Despite recent decline in malaria consultation rates, malaria was a problem in Kersa. Furthermore results presented in this study suggest that the burden of malaria in children <5 years of age is still significant. Our assessment indicated that annually, malaria consultations peaked during September to December which coincides with the end of the rainy season.

340

PLASMODIUM KNOWLESI TRANSMISSION IN ANDAMAN AND NICOBAR ISLANDS OF INDIA AND DRUG RESISTANCE GENOTYPES

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Recent studies in Southeast Asia have shown zoonotic transmission of *Plasmodium knowlesi* to humans. It was not known whether *P. knowlesi* transmission occur in India. Recently, we investigated the possible presence of *P. knowlesi* in Andaman and Nicobar group of Islands as these islands are situated in the Bay of Bengal in an ecological zone similar to Southeast Asian countries such as Malaysia and Indonesia where transmission of this parasite has been shown. This was a retrospective investigation of clinical samples obtained from previous studies involving several islands in this region. In this investigation we sequenced the chloroquine resistance transporter(CRT) and dihydrofolatereductose (DHFR) genes of *P. knowlesi* and other *Plasmodium* species. The merozoite surface protein-1 and 18S rRNA genes of *P. knowlesi* were also sequenced from these samples. Among 445 samples analysed , only 53 of them had P. *knowlesi*-specific gene sequences.While 3 of the (86.79%,n=46)or *P. vivax* (7.55%,n=4) but none with *P. malariae* or *P. ovale* . All *P. knowlesi* isolates contained wild type sequences of crt and dhfr genes while *P. falciparum* isolates had mutation in CRT and DHFR marker genes. The mutation pattern indicates that the same patient having a mixed infection may be harbouring the drug susceptible *P. knowlesi* parasite and a highly drug resistant *P. falciparum* parasite. The implications of these findings in the context of evolving drug resistance and treatment strategies including ecological changes associated with transmission of *P. knowlesi* will be discussed.

341

POPULATION STRUCTURE OF *PLASMODIUM VIVAX* IN AN URBAN VILLAGE OF THE PERUVIAN AMAZON (SAN JUAN-IQUITOS)

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In Latin America, Peru is among the countries with the highest malaria burden, mainly due to *Plasmodium vivax* infections. However little is known about *P. vivax* genetic structure, which is essential to describe the transmission dynamics, in the Peruvian Amazon where most of the malaria cases occur. Hereby we determined the genetic diversity and population structure of *P. vivax* isolates collected in the Peruvian Amazon. A total of 65 *P.vivax* patients recruited in San Juan city (Iquitos, Peru) between April, 2008 and February, 2009 were treated radically with chloroquine and primaquine and followed up monthly for 2 years with systematic blood sampling. All samples were screened for malaria parasites by microscopy and PCR, subsequently all *P. vivax* infections were genotyped using 15 microsatellites. Parasite population structure and dynamics were determined by computing different genetic indices. *P. vivax* population structure was determined by multilocus genotyping using 15 microsatellites on 297 *P. vivax* infected blood samples collected. The genetic diversity was determined by calculating the expected heterozygosity (He). Both linkage disequilibrium and the genetic differentiation were estimated. The population characteristics were assessed only in samples with monoclonal infections (n=242). The proportion of polyclonal infections was 10.7%. The *P. vivax* populations circulating in San Juan city are genetically diverse however they have a low recombination rate. Clonal parasite reproductive events in this area are indicated by the presence of significant linkage disequilibrium. The low malaria transmission may favor as well the clonal parasite population.

342

EXPLORATION MALARIA AND OTHER VECTOR-BORNE ILLNESSES INCIDENCE IN ENTOMOLOGICAL WORKERS

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Mosquito collections are vital to surveillance certain vector-borne illnesses (VBI). Human-baited collections (HLC) remain the only reliable method to assess the presence and behavior of anthropophilic malaria vectors. However, HLC is considered to increase the risk of acquiring malaria, although empirical evidence from low-endemic settings is lacking. We explored perceived and actual risks associated with HLC in the Peruvian Amazon Basin, a low-endemic malaria setting, through a longitudinal study of entomological workers (EW) from Iquitos. Malaria/VBI incidence and exposure were determined every three months by microscopy, nested PCR and enzyme-linked immunosorbent assay. Signs/symptoms, work-conducted practices, and behaviors were assessed monthly. A focus group was conducted to understand the knowledge and perceptions of EW-associated risks. We enrolled 19 EW (males=89%, mean age=37 years, mean EW time=9 years), who qualitatively identified themselves as study personnel. Their HLC-related self-perceived risks were: VBI, rabies, dog/sneak bites, traffic accidents, and working in narco-terrorism affected areas. HLC was carried out by 84% (n=16) of EW, 61% as their regular activities. The self-reported lifetime prevalence of infectious diseases was: malaria (68%), diarrhea (68%), dengue (53%), leishmaniasis (5%), hepatitis (5%), and Oropouche fever (6%). Protective measures used were: long pants (97%) and sleeves (72%), mosquito repellent (20%), head nets (20%) and insecticide-impregnated clothing (5%). All study participants were malaria-free at baseline, 15% exhibited circulating IgG antibodies to PvMSP-1, 84% to dengue virus, and 21% to other arboviruses. Four participants had malaria over the 10 months of study (169 person-months) representing an incidence of 2.3 cases per 100 person-months in this population. All malaria free individuals (n=15) remained seronegative to PvMSP-1_{19} from baseline until the end of follow-up. Out of 86 HLC-months, only 5% resulted in malaria infections. EW are a heterogeneous group with diverse behaviors and preventive practices. These findings indicate that additional studies need to be conducted with and adequate comparison group to determine if HLC puts EW at increased risk for malaria/VBI even in low endemicity settings. As research staff, EW should be provided with proper education and occupational health support to minimize any VBI risks.

343

TRANSMISSION OF MALARIA: EPIDEMIOLOGICAL AND CLINICAL PROFILE OF SEVERE MALARIA IN ADULTS IN KINSHASA (2007-2012)

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Malaria is endemic in the tropics. The age group most exposed to severe forms is that of children aged 0 to 5 years. Due to natural immunity, adults are protected and rarely develop serious forms of malaria. Nowadays, more and more cases of adults in the severe form of malaria are reported. The objective of our study was to describe epidemiological and clinical profile of severe malaria in adults in Kinshasa. 148 cases from 9757 patients admitted to infectious disease clinic or 1.5% of all cases of hospitalization showed the severe form of the malaria. The female sex was predominant with a sex ratio of 1.06. The age group most struck was that of 18-34 years (35.8%). Fever was present in 64.9% of patients and pale mucous membranes and hemoglobinuria were respectively found in 12.2% and 4.1% of cases. The *parasitemia* was observed in 44% of patients. Cerebral malaria has been reported in 71.4% of cases. Quinine intravenous and intramuscular artemether were administered respectively in 95.5% and 4.1% of cases the outcome was favorable in 130 patients (87.8%). There were 18 deaths (12.2%). In conclusion, although rare (1.5%), severe malaria remains a reality in Kinshasa and should be described to take effective control measures.

344

NOVEL SEROLOGIC ASSAYS OF *PLASMODIUM FALCIPARUM* EXPOSURE

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Assessing *Plasmodium falciparum* (Pf) exposure is labor intensive and inaccurate. Serologic assays offer promise of greater precision at low cost, but appropriate antigens against which to assess responses representative of exposure are uncertain. Kinetics of Pf-specific antibody responses differ by antigen, suggesting that appropriately selecting antigens for antibody assessment will increase accuracy of Pf exposure estimations. Analysis of Pf protein microarrays probed with plasma from Malian children, aged 2-10 years, identified antibody kinetics predicting cumulative and recent

exposure. Based on these analyses, we probed a smaller array with plasma collected at 4 years of age from 79 children in Tororo, Uganda, where malaria transmission intensity is very high (entomological inoculation rate >300). Subjects had been followed from ≤10 months of age with continuous passive surveillance and monthly blood smears. Cumulative exposure markers were identified by linear fit of antibody intensity vs. total number of malaria episodes. Recent exposure markers were those that best predicted time since last *parasitemia*, assuming exponential antibody decay. Of 39 cumulative and 34 recent exposure markers selected in Malian children, 20.5% and 32.4% respectively were also predictive of exposure in Ugandan children. Cross-sectional analysis of responses to erythrocyte stage antigens AMA1 and MSP1 are commonly used to assess Pf exposure. However, in our age-restricted cohort, the highest R2 for the 13 AMA1 and MSP1 antigen fragments on our array was 0.08. In contrast, PfEMP1, MSP11, and ETRAMP4 R2 ranged from 0.34-0.40. Evaluated together, these 3 antigens gave a cumulative exposure R2 of 0.52. Serologic responses to ACS5 and ETRAMP4 predicted time since the last parasitemic episode with an R2 of 0.65, allowing estimation of recent exposure. Though this new approach toward predicting Pf exposure demonstrates some variance for individuals, it offers promise for precise population estimates. We are currently evaluating responses across multiple transmission settings and age ranges in Ugandan children to further generalize these findings and to develop robust models of Pf exposure.

345

MALARIA MORBIDITY AND PREVALENCE OF INTESTINAL PARASITES DURING THE FIRST YEAR OF LIFE IN RURAL SENEGAL

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Malaria, the first parasitic endemic disease throughout the world, touches especially in Africa in the south of the Sahara, the children of less than five years. In Senegal, the various fight plans, involved a clear reduction among case of malaria which passed from 408,588 to 30,800 between 2006 and 2009 in the children of less than five years. Thus in front of the frequency of malaria, anaemia and the intestinal parasitic bearing in the children of less than 5 years, we proposed more specifically to study them in the infants up to one year where data are fewer. We conducted a longitudinal prospective descriptive study of follow-up of a cohort of children since the age from 4 to 6 weeks during one year. The follow-up visits were monthly. The subjects of the study were also visited in a weekly way in residence for early detection of the cases of malaria. The rate of haemoglobin was measured with a device Hemocue Hb 301. An examination of saddles in a fresh state was carried out .The study protocol was subjected and approved as a preliminary by the national ethics committee of Senegal. At the end of one year follow up visits for participants(from November 2010 to March 2012), one clinical malaria at 59 days of age confirmed by finger prick thick (1640 Pf/µl) was observed. The prevalence of malaria in our study was 0.7% (1/138) by taking account of the 12 withdrawals of study. No case of malaria infection was noted. The mean haemoglobin was 12.4 mg/dl at inclusion. The moderate anaemia was 16.7% at inclusion and had appreciably increased to 55.3% at 6months and 51.9% at 12 months. The prevalence of the bearing for the principal intestinal parasitic species found in our study is the following one by order of importance: *Giardia intestinalis* (15.4%), *Ascaris lumbricoides* (8.5%), *Entamoeba coli* (1.5%), yeasts (1.5%), *Trichuris trichiura* (0.8%). In our cohort of study the acute respiratory infections dominated in the reasons for consultation. Indeed 94.6% of the children came to consult at least once for an acute respiratory infection. Two deaths were noted during follow up for breath infections. Preliminary results of this study confirm that infants are protected during their first months of life. These results were reinforced because all participants of the study were followed after the period of intensive transmission between September and November.

346

MALARIA TREATMENT SEEKING BEHAVIOR: FACTORS INFLUENCING CARE SEEKING FROM SIMILAR/EQUAL LEVEL, RURAL AND MORE DISTANT FACILITIES

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Distance greatly affects health care access. In rural, high malaria endemic Uganda (562 infective bites per person per year) with an unreliable transport system, some patients walk, cycle, or ride pillion on a motorbike, to more distant lower level government run health facilities (PHF) for care. In this study we explore the main factors compelling caregivers and/or patients to travel to further PHF for care and thus, provide a framework for improvement of patient centered care advantaged by proximity. We collected standard HMIS out patient data (OPD) from20 PHF in a cluster randomized trial with 10 in the health facility intervention (HFI) and 10 in standard care (SC), in 7 sub-counties of Tororo district. OPD was collected from Apr-2011 to date and we conducted spatial analysis on 14 months of the data to examine patients' residence in relation to the PHF they visited. 263,873 patients with mean age of 17.9yrs were seen, 31.6% being under-fives. We defined distance traveled as the Euclidean distance -centroid of a patient's village of residence to the PHF visited- categorized as: <2km; and, >2km. We quantitatively analyzed OPD for influence of: level of PHF, Intervention arm, age, gender, malaria diagnosis, and recorded fever history. Also, qualitative data in the study was analyzed for patients' motivations in choosing PHF. Preliminary results suggest: 40.4% of patients travel >2Km to a PHF; odds in traveling >2Km for HFI to be 22% higher than for SC, adjusting for health facility level, gender, malaria diagnosis and age; odds for under-fives are 8% higher than for older patients, adjusting for other factors; odds for patients with no malaria diagnosis are 5% higher than for those with a malaria diagnosis, adjusting for the other factors; and, No statistical difference between male or female, or having a history of fever or not. Testing services were cited as a major reason to travel further. We posit this explains traveling further to HFI -offering malaria RDT testing- versus SC. We will present full results with the full data and other dynamics over time.

347

THE EFFECTS OF URBANIZATION ON MALARIA METRICS IN UGANDA

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Sub-Saharan Africa is expected to show the greatest rates of urbanization over the next 50 years. Urbanization has shown a substantial impact in reducing malaria transmission, because, amongst other factors, urban areas generally provide unfavorable habitats for *Anopheles*, and urban populations are generally healthier and have better access to healthcare. Statistical relationships have been explored at global and local scales, but only examining the effects of urbanization as a binary variable, and only looking at the effects of urbanization on a single malaria metric. Here we undertake the first analysis that examines the impact of varying degrees of urbanization on a variety of malaria metrics. Cohorts of 100 households (HH) across three contrasting districts of Uganda (Jinja, Tororo and Kanungu) were followed for a 11/2 year period. For each district and HH, *Anopheles* catches were measured, Entomological Inoculation Rates (EIR) were calculated, parasite rates (PR) were measured and clinical metrics were gathered. Measurements of the intensity of urbanization in the vicinity of each HH were also calculated through HH density and satellite imagery based metrics. Correlation analyses between the variety of urban intensity and malaria metrics were then undertaken. Consistent and significant correlations between urban intensities and malaria metrics were found. HH density and urban land cover proportion measured through

satellite imagery classification both showed significant correlations with decreased numbers of *Anopheles*, EIR, PR and clinical incidence. These differences were much clearer for Jinja district, which showed the greatest range in levels of urbanization of the three studied. These results highlight the substantial impact of urbanization on malaria. With reduced numbers of mosquitoes, lower prevalence and lower clinical incidences, urban areas are shown to have a consistent impact on several malaria metrics. With increasing urbanization rates, results here point to continued declines in rates of malaria across Africa.

348

DECREASED OCCURRENCE OF HIGHLAND MALARIA AFTER INTRODUCTION OF POINT-OF-CARE RAPID DIAGNOSTIC TESTS IN KENYAN HIGHLAND NEAR ELDORET IN 2250 METERS ABOVE SEA LEVEL

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Malaria is known to infect people also in altitudes higher than 2000 m above sea level (a.s.l). Several cases of *falciparum* malaria have been already described from Ethiopian and Kenyan highlands in 2200 m a*.s.l.* Rapid diagnostic tests (RDT) to confirm microscopically positive cases or even to diagnose malaria without microscopy has been shown to be highly sensitive, enough to diagnose malaria and to initiate necessary semi- empiric therapy of slide-positive fever. Eldoret is lying in Cherangani Hills in high of 2200 - 2290 m a*.s.l.* and Ladislaus Batthyanyi -Strattmann Clinic which is located there serves medical services for urban population of around 100000 people and it treats 25000 - 30000 patients per year. The aim of this study was to differentiate if slide-positive cases of fever in altitude of Eldoret highlands (2250 - 2500 m a*.s.l.*) can be confirmed with RDTs as highland malaria. Annual occurrence of malaria has been compared in two periods: (i) before introduction of RDTs (2009-2010) and (ii) after (2011-2012) RDTs have been introduced in combination with microscopy for suspected malaria, especially in patients with travel history to Kenyan down country.From January 2009 to May 2012, 16181 cases of microscopically positive malaria have been diagnosed. In 2009, 7760 cases and in 2010, 5006 cases were diagnosed microscopically. When RDTs were introduced in January 2011, in combination with blood smear microscopy in our laboratory, the number of confirmed cases dropped to 1919 in 2011 and 748 in first six months of 2012, which is 2,5 to 3-times (80%) less than in previous years. In this study, we proved that introduction of point-of-care RDTs into malaria diagnostics in combination with blood smear microscopy led to significant decrease of highland malaria diagnosis as well as artemisinin-based combinational therapy mis-dosing in Kenya.

349

HIGHLAND MALARIA IS NOT RARE BUT SHOWS DECREASING TENDENCY: FOUR YEARS OF FOLLOW UP IN MURAGO HOSPITAL, BURUNDI

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Highland malaria used to be an underestimated entity, however now we know that malaria due to global warming exists also in areas higher than 2000 meters above sea level (a.s.l.). We have seen number of cases of highland malaria in Rwanda, Burundi, Kenya and even in Murago located above 2500 above sea level (a.s.l.). In this study, we have investigated incidence of malaria in Eldoret (2200 meters a.s.l.), Kenya, during four years 2009 - 2012. Diagnosis of malaria was assumed according to clinical signs, blood smear microscopy and positivity of rapid diagnostic test (RDT). We have observed decreasing number of malaria cases at St. Ladislaus Strattmann Clinic, as 7760 patients in 2009, 5006 patients in 2010, but only 1919 patients in 2011 and 1210 patients in 2012 were diagnosed there with highland malaria. Explanation for decreasing number of highland malaria cases may be decline of travel to down country which is probably result of economic crisis. Hereby, we can debate about influence of another economical factor on spread of malaria.

350

A SET OF NOVEL POLYMORPHIC TANDEM REPEATS MAKERS FOR *PLASMODIUM VIVAX*

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Enhanced understanding of the transmission dynamics and population genetics for *Plasmodium vivax* is crucial in predicting the emergence and spread of novel parasite phenotypes with major public health implications, such as new relapsing patterns, drug resistance and increased virulence. Suitable molecular markers are required for these population genetic studies. Here, we focus on the variable number of tandem repeats (VNTRs) which provides valuable information about both the functional and evolutionary aspects of genetic diversity. Although polymorphic microsatellite markers had been optimized in *P. vivax* and used to analyze the population structures of *P. vivax* in many countries, the VNTR based markers and polymorphism have not been examined. In this study, we optimized a set of VNTR markers from 253 *P. vivax* ORF genes and analyzed by Sequence-based Estimation of Repeat Variability (SEVR, analyze DNA sequence and provide "VAR" score). The top 6 of them were used to screen 83 parasite isolates from Southeast Asian countries (China, Korea and Thailand). The total number of alleles per locus ranged between 4 and 9, and the mean of expected heterozygosity (HE) in China, Korea and Thailand are 0.54, 0.51 and 0.72, respectively. Significant linkage disequilibrium was maintained. Population structure showed strong clustering of outbreak isolates from central China and South Korea was observed. Results showed that the genetic variability of 3 populations using these 6 TR makers was similar to those previously reported markers. Furthermore, population structure investigation by using these TR makers has revealed that Chinese (central) and South Korean have similar population structure which was clear different from Thai. We deduce that these molecular markers can be used to characterize the population structure of *P. vivax* in other endemic areas.

351

FINE MAPPING OF THE CD36 BINDING SITE FOR *PLASMODIUM FALCIPARUM* PARASITIZED ERYTHROCYTES

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CD36 is a conserved scavenger receptor that interacts with a diverse number of ligands including *P. falciparum* parasitized erythrocytes (PEs) and oxidized LDL (oxLDL). The crystal structure of LIMPII (Lysosomal

Integral Membrane Protein II), a Class B Scavenger protein closely related to CD36, has recently been solved. Using this structure as a template, the 3D structure of human CD36 (hCD36) was modeled and predicted to exist as a homodimer (dimerization region amino acids 151-181). To test these predictions several mutations were introduced in order to break this secondary structure. The constructs were engineered as GFP fusions and transiently transfected into HeLa or COS-7 cell lines. Transfected constructs were tested for binding of oxLDL and PEs. Binding was quantified and normalized by enumerating only GFP expressing cells. Surface expression of the constructs was determined by immunofluorescence using an anti-hCD36 antibody and a proteinase K protection assay of intracellular proteins. Mutations introduced in this region abrogated binding of both ligands. Residues L158, L161 and in combination K164/K166 seemed to be crucial for maintaining the integrity of the binding site supporting the homodimer model theory. It has been believed that the CD36 binding site for PEs is similar to oxLDL. By studying the binding affinity of different highly conserved CD36 orthologs we found that bovine CD36 does not bind PEs but does bind oxLDL. Interestingly, residues L158, L161, K164 and K166 are highly conserved across orthologs. We hypothesized that a discrete highly polymorphic overlapping region (aa 146-156) might encode the observed differential PE binding. Here we show through detailed site directed mutational analysis that the binding sites for PEs and oxLDL are distinct. Further, ortholog swap analysis between bovine and hCD36 is being used to fine map the PE binding site by defining the minimal requirements to confer PE binding to bovine CD36 and conversely abrogate PE binding by hCD36, while preserving oxLDL binding.

352

CHARACTERIZATION AND ANTIGENICITY OF *PLASMODIUM VIVAX* RHOPTRY-ASSOCIATED LEUCINE ZIPPER-LIKE PROTEIN 1 (PVRALP1), A NOVEL RHOPTRY NECK PROTEIN

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Rhoptry secreted proteins are associated with tight junction and parasitophorous vacuole formation during invasion of host targets cells and are sorted within rhoptry neck or bulb. We have identified blood-stage antigens of *Plasmodium vivax* likely to be highly immunogenic. Of these candidates, a novel protein PVX_096245, which is the ortholog of rhoptry-associated leucine zipper-like protein 1 (RALP1) from *P. falciparum*, was gained for detailed characterization. PvRALP1 contains a novel glutamate(Glu)/glycine(Gly)-rich domain that is conserved in other *Plasmodium* species. In present study, full-length without signal peptide (Ecto) as well as a Glu/Gly-rich domain (Tr) of recombinant PvRALP1 were expressed by using cell-free expression system. Sera screening experiments indicate that PvPvRALP1-Ecto and PvRALP1-Tr possess 58.9% and 55.4% in sensitivity and 95.0% and 92.5% in specificity. The PvRALP1 is localized in rhoptry neck; an apical organelle of the merozoite, and the localization of this protein is firstly defined in *P. vivax* . Of PvRALP1 immunogenicity, cytophilic antibodies were produced simultaneously. The present study suggests that PvRALP1 is immunogenic in humans during parasite infection and it may be a novel potential vaccine candidate in the blood stage of *vivax* parasite.

353

PATTERNS OF GENE FLOW IN *PLASMODIUM VIVAX* POPULATION CURRENTLY CIRCULATING IN SRI LANKA - A COMPARATIVE GENETIC POPULATION BASED STUDY

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Epidemiological evidence of relatively unstable and low intensity malaria transmission due to successful elimination strategies lead Sri Lanka to achieve malaria pre-elimination status in the year 2008. Understanding the population genetic structure of current and previous local *Plasmodium vivax* isolates is important to (i) examine the degree of genetic isolation of these populations, and (ii) ascertain whether subsequent outbreaks would be due to residual transmission or due to introduction of new parasite strains to the parasite population, enabling population specific malaria control measures Sequences of *P. vivax* isolates that circulated locally, a decade ago was obtained from the Genebank (pvmsp3α: N=17; pvdbpII=100, pvmsp142= 95, pvcsp: N=60). PCR amplification and sequence analyses of these four polymorphic *loci* of *P. vivax* were carried out using 16 samples collected recently (2011-2012). Expected heterozygocity (He) and the genetic differentiation (Fst) was examined using DNasp 5.1 software, to draw comparison of current and previous population genetic structures. Low mean (He) in the current *P. vivax* population (He=0.76) compared with the previous population (He=0.93) was observed for all four genes indicating less gene diversity in the currently circulating isolates. However, the He of pvmsp-142 (0.962) current population was higher than that of the previous (0.978). Genetic differentiation (Fst) between the two test populations was highest in pvmsp3α (0.20719) followed by pvcsp (0.1271) indicating great differentiation between the two populations. PvdbpII (0.0793) and pvmsp-142 (0.0018) showed moderate and little genetic differentiations, respectively. Linkage disequilibrium was maintained across the current population except for pvmsp3α. A reasonable degree of overlap of amino acid haplotypes in these four proteins and not many novel a.a haplotypes were observed between current and previous populations. Thus these results for the first time in Sri Lanka suggest that new *P. vivax* variants may have been introduced to the island with simultaneous residual transmission of previously detected alleles. Further investigation is needed in order to ascertain the risk of re-introduction.

354

HIGH RESOLUTION MELTING: AN ADAPTABLE AND DEPLOYABLE METHOD IN THE FIELD TO MONITOR *PLASMODIUM FALCIPARUM* GENETIC POLYMORPHISMS

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Understanding the genetic variation in malaria parasite populations and how selective pressures such as drug treatment regimes alter these patterns of genetic variation can be used to identify molecules responsible for changes in drug response, and to develop tools that can provide an early warning system for the emergence of drug resistance when new anti-malarial drug pressure is applied. These tools should be fast, sensitive, unambiguous, and cost-effective, and deployable in malaria endemic field sites. We have successfully deployed High Resolution Melting (HRM) technique in Senegal to analyze molecular four genes pfcrtK76T,

pfmdr184/86/1042, dhfr51/59/108/164 and dhps437/540/581/613 in 27 parasite samples from ACT *in vivo* efficacy study performed in Thies, Senegal. The prevalence of pfcrtK76T mutant and mixed alleles are: 33.33% and 11.11% mixed; 96.30% and 3.70% mixed for dhfrN51I; dhfrC59R (92.6% and 3.70%); 92.59% and 7.41% for dhfr108N; and 100% wild type dhfr164I. For the dhps gene we have 100% wild type in *loci* A581G and K540E; 55.56% mutant and 11.11% mixed for A437G; 3.70% mutant allele in *loci* A613T/S. For pfmdr, all parasites were wild type at N1042D, but in *loci* Y184F we found 55.6% mutant and 3.7% new sequence; 88.9% wild type and 11.1% mutant for the *loci* N86Y. Our results illustrate that the HRM is an adapted and deployable method in malaria endemic countries to track parasite genetic polymorphisms in real time.

355

EPIGENETIC CHANGES IN THE DEVELOPMENT OF CHLOROQUINE RESISTANT PHENOTYPES OF *PLASMODIUM FALCIPARUM*

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The current scope of chemotherapy in the treatment of malaria has been limited due to the development of drug resistance to a number of highly effective drugs and combinations including chloroquine (CQ). Mutations in the *Plasmodium falciparum* gene *loci* Pfmdr1-N86Y and Pfcrt-K76T are known to confer drug resistance to CQ associated with long term use. However, variable phenotypic effects that mediate drug sensitivity reversal can be caused by epigenetic mechanisms via chromatin remodeling and DNA methylation that affects the state of gene activation and silencing thereby altering the levels of gene expression. In this investigation, we seek to identify the epigenetic changes that mediate altered drug resistance levels to chloroquine. This will involve *in vitro* stepwise selection of *P. falciparum* strains exposed to different concentrations of CQ and assessing for reduced drug sensitivity via a SYBR Green I-based *in vitro* IC_{50} drug sensitivity assay. Variations in gene copy number will be validated via qReal Time-RT PCR for Pfmdr1 while gene mutations in various codons of Pfmdr1 and Pfcrt will be accessed via RFLP-PCR. Global epigenome signatures associated with the histone modification will be investigated via chromatin immunoprecipitation (ChIP) and DNA methylation by ELISA at these particular *loci*. Comparisons will be made with the controls which consist of the unexposed *P. falciparum* strains. This investigation aims at providing further information into the molecular evolutionary mechanisms of CQ anti-malarial drug resistance.

356

POPULATION GENETIC STRUCTURE, AT A SPATIAL AND TEMPORAL LEVEL, OF *PLASMODIUM VIVAX* IN THE PERUVIAN AMAZON BY GENOTYPING WITH THE QIAXCEL ADVANCED SYSTEM

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Iquitos, the most populated and biggest city in the Peruvian Amazon, is well known for being a hypoendemic region for *Plasmodium vivax*. Consequently, there is little information about the transmission dynamics of *P. vivax* in this region. Therefore, the purpose of this study was to determine the genetic diversity, at a spatial and temporal level, of *P. vivax* in the Peruvian Amazon by genotyping with 15 DNA microsatellites with the QIAxcel Advanced System from QIAGEN. 270 *P. vivax* infected blood samples, which were collected through passive surveillance between the communities of Santo Tomás, San José de Lupuna, and Padrecocha in the city of Iquitos, were genotyped with 15 DNA microsatellites with the QIAxcel Advanced system. The analysis of allelic variation of the monoclonal isolates with complete profiles showed that out of the 15 DNA microsatellites, MS16, MS12, MS8, MS9, and Pv6635 were the most polymorphic ones as defined by their Hunter-Gaston Discrimination Index (HGDI). This makes them the most indicative ones for determining population genetic structure of *P. vivax* in the Peruvian Amazon. The analysis of the complete profiles demonstrated that the vast majority of samples in each of the three sites had unique profiles. In Padrecocha, 60 out of 63 samples had different profiles, in San José de Lupuna, 57 out of 60 had different profiles, and in Santo Tomás 36 out of 39 samples had different profiles. The analysis by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) showed that out of the 162 isolates with complete profiles, there were 9 different groups; each of these could have had descended from a common ancestor. An analysis with the BURST algorithm (Based Upon Related Sequence Types) presented a total of 24 groups that had 13 out of 15 identical *loci*. 75 isolates were considered singletons. The 270 *P. vivax* isolates found in the Peruvian Amazon showed to be genetically diverse, both at a spatial and temporal level, since the analysis by profile determined unique profiles for the vast majority of the isolates. The BURST analysis supports this idea giving 75 singletons out of the 162 isolates with complete profiles.

357

POLYMORPHISM -1447A>G IN CXCL10 GENE PROMOTER SEQUENCE IS ASSOCIATED WITH INCREASED EXPRESSION LEVEL OF CXCL10 AND CEREBRAL MALARIA PATHOGENESIS

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Cerebral malaria (CM) is a neurological complication of *Plasmodium falciparum* infection and a major cause of mortality in children under 5 years of age. Several host and parasite genetic factors have been implicated in CM pathogenesis. The risk factors for CM and the wide variation in clinical manifestations of malaria are poorly understood. Human genetic variation has been shown to influence susceptibility to malaria, progression to CM and death. Recent studies have shown CXCL10, an angiostatic and pro-apoptotic chemokine, to be a strong predictor of both human CM and experimental CM. Increased plasma and cerebrospinal levels of CXCL10 was tightly associated with fatal CM in humans in India and Ghana. Furthermore, it has been demonstrated that increased CXCL10 production in cerebral malaria patients is responsible for inducing apoptosis in brain vascular endothelial and glia cells thereby causing blood-brain barrier dysfunction and damage. In the present study, we hypothesized that in a subset of malaria patients, CM is genetically linked to variation in plasma CXCL10 expression. We wanted to determine whether published polymorphisms in the CXCL10 gene promoter region played a role in the clinical status of malaria patients and address the genetic basis of CXCL10 expression during malaria infection. Two known SNPs in the CXCL10 promoter (-1447A>G and -135G>A) were selected on the basis of their association with CNS disorders and genotyping was performed in 66 CM and 69 non-CM patients using PCR-restriction fragment length polymorphism assay. We found that the -1447A>G polymorphism was significantly associated with susceptibility to CM. Individuals bearing at least one G allele of the -1447A>G polymorphism were susceptible to CM (OR = 2.60, 95% CI = 1.51 - 5.85, p = 0.021). Moreover, individuals with the A/G genotype (-1447A>G polymorphism) had significantly higher serum CXCL10 levels than the AA genotype. The A to G substitution in the -1447A>G polymorphism resulted in the loss of binding site for TATA-binding protein and interferon regulatory factor 4. However, we did not find any association between the -135G>A

polymorphism and CM. Polymorphisms in the CXCL10 gene promoter sequence was associated with high CXCL10 production, which plays a role in severity of CM. These results suggest that the -1447A>G polymorphism in the CXCL10 gene promoter could be partly responsible for the genetic variation underlying susceptibility to CM.

358

FOLATE PATHWAY POSSIBLY ASSOCIATED WITH *PLASMODIUM VIVAX* RELAPSE IN THE PERUVIAN AMAZON BASIN AFTER PRIMAQUINE TREATMENT

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Primaquine (PQ) is used to prevent *Plasmodium vivax* (PV) relapses by eliminating hypnozoites. Two hypothetical mechanisms of action of PQ are thought to be oxidative stress and pyrimidine synthesis inhibition. PV relapses after PQ therapy are suspected to involve complex host and parasite factors, but due to the lack of PV culture assays there is limited empirical evidence. Therefore we studied the association between relapses and mutations in 3 genes encoding folate pathway enzymes, which are precursors of pyrimidine synthesis pathway, and a transporter protein that could be involved in the detoxification process. We studied parasite genetic factors and clinical-epidemiological data associated with relapse, conducting a case-control study within a clinical trial that investigated the efficacy of 3 different doses of PQ to prevent relapses. This study was conducted from 2006 to 2008 in 3 communities in the Peruvian Amazon Basin: Padrecocha, San Juan and Santa Clara. The main result showed that the two study arms with total doses of 210 mg had lower 6-month relapse rates than the arm with total dose of 150 mg. We included 47 individuals who had a PV homologous relapse (relapse cases). The 401 individuals who did not experience a relapse were included as controls for clinical-epidemiological factors (non-relapse controls). DNA sequencing was performed to identify mutations in the *Pvdhfr*, *Pvdhps* and *Pvmdr1* genes in all cases and a random sample of control subjects (n=57). Subjects with weight>58 Kg (HR 2.71, *p=0.012*) and living Padrecocha and San Juan (HR 3.37 *p=0.033* and HR 2.89 *p=0.047* respectively) had increased relapse risk, after adjusting by treatment arm and age. The A383G mutation in the *Pvdhps* gene was associated with more frequent relapses (38% vs 19%, *p=0.032)*. Also, the triple mutant *Pvdhfr* genotype F57/S58R/Y69(TAT>TAC)/S117N was more frequent in relapses than controls (62% vs 38%, *p=0.033)*. *Pvmdr1* was not associated with relapses. The association with *Pvdhfr* remained marginally significant after adjusting by community and weight, despite the small sample size.These findings suggest that mechanisms in folate pathway of the parasite could be involved in relapses after PQ treatment, possibly as a pyrimidine synthesis modulator. More studies are needed to validate these results.

359

CYCLIN-DEPENDENT KINASE PFMRK PLAYS AN IMPORTANT ROLE IN G1/S PHASE TRANSITION IN *PLASMODIUM FALCIPARUM* CELL CYCLE

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Clinical symptoms of malaria result from the rapid growth and cyclic multiplication during erythrocytic schizogony. This process is controlled by the parasite cell cycle regulatory mechanism. A better understanding of this parasite cell cycle regulatory mechanism may lead to means to interrupt parasite growth and replication; and development of novel antimalarial drugs. Cyclin dependent protein kinases (CDKs) are major regulators for growth and proliferation of mammalian cells. Pfmrk, a sequence homologue of human CDK7 is suggested to play an important role in parasite cell cycle regulation. We investigated the role of Pfmrk in both cell cycle regulation and DNA replication in the plasmodial cell cycle using transgenic parasites that over-express functional Pfmrk (HPG), non-functional Pfmrk (HKG) and control (empty vector control). We observed that HPG has accelerated the transition of trophozoites to schizonts while this transition was delayed in HKG. This accelerated transition in HPG was blocked by WR636639, a second-generation chalcone that selectively inhibits Pfmrk, when added to ring-stage parasites. In contrast, when WR636639 was added to trophozoite-stage parasites, the transition from schizonts to rings was delayed. A similar inhibitory effect was also observed in the HKG line.These findings suggest that Pfmrk plays an important role in the transition of G1/S phase, parasite growth and development.

360

MEASURING ERYTHROCYTE SURFACE-ANCHORED PFEMP1 LEVELS AMONG PROGENY FROM A 3D7 X HB3 *PLASMODIUM FALCIPARUM* GENETIC CROSS

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Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) is the main parasite virulence factor of *P. falciparum* due to its central role in mediating the cytoadherence of infected red blood cells (iRBCs) to the host microvascular endothelium during parasite infection. Directly targeting PfEMP1 as a therapeutic strategy is greatly limited due to the protein's hypervariable nature, which gives rise to approximately 60 different variants. Interfering with the trafficking of PfEMP1 to the iRBC surface is a more attractive therapeutic approach, as hypervariability becomes inconsequential and previous studies have demonstrated that reduced surface PfEMP1 levels significantly weaken cytoadherence, which likely lessens the severity of malaria symptoms. Interestingly, the *in vitro* culture-adapted parasite line 3D7 is inherently defective in exporting PfEMP1 to the iRBC surface. Presuming that PfEMP1 export from the parasite to the iRBC surface is controlled at the genetic level, we hypothesized that 3D7 harbors one or more genetic determinants of impaired PfEMP1 trafficking. To test this, we examined the surface PfEMP1 levels of 17 progeny clones obtained from a genetic cross between 3D7 and the 'trafficking-competent' parasite line HB3. This was accomplished using both Western blotting and a two-color, triple-layer flow cytometry assay with plasma from malaria-immune Malian adults. We found that 3D7 displays 75% less PfEMP1 on the iRBC surface than HB3. Progeny phenotypes normalized to

HB3 range from 37% more to 88% less surface PfEMP1 levels. Using these phenotypes in QTL analysis, we identified significant *loci* on chromosomes 12 and 14 that each explain 50% of the phenotype variance. The role of candidate genes in the trafficking of PfEMP1 to the iRBC surface will be confirmed in allele-exchange experiments, where the defect is rescued in 3D7 and introduced in HB3. The results of this study may strengthen our understanding of malaria pathogenesis and provide new targets for much needed therapeutics.

361

HAPLOTYPIC VARIATIONS IN HSP70 ARE ASSOCIATED WITH ALTERED EXPRESSION OF HSP70, INFLAMMATORY MEDIATORS, AND SUSCEPTIBILITY TO SEVERE MALARIAL ANEMIA

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The present study was designed to validate a genome-wide association study (GWAS) and transcriptome data generated using 'polarized' samples from children infected with *Plasmodium falciparum* malaria (n=48; 3-36 mos.). Although these experiments resulted in the identification of several important genes, here we report the association between markers in the heat shock proteins (Hsp) 70 encoding genes and severe malarial anemia [SMA, hemoglobin level <5.0 g/dL]. Hsp70 is a major stress-inducible protein that acts as a chaperone, immunomodulator, and mediator of antigenic peptide delivery and presentation. The HSP70 gene family is comprised of two nearly identical inducible HSPA1A and HSPA1B genes. Variation in these genes has been implicated in the pathogenesis of several diseases, however, a role in malaria has not been reported. As such, we validated the markers identified in the GWAS [i.e., HSPA1A (-217C/G; -5457A/C; -5893G/A) and HSPA1B (-4439G/A and -8133T/C)] and examined their association with SMA in parasitemic children (n=854). Binary regression analyses controlling for confounders revealed that carriage of the CAGGT, CAAGT, and CAAAT (-C217G/-A5457C/-G5893A/-G4439A/-T8133C) haplotypes were associated with reduced risk of developing SMA [(odds ratio (OR), 0.50; 95% CI, 0.25-0.96; P=0.034) (OR, 0.27; 95% CI, 0.10-0.68; P=0.006) and (OR, 0.36; 95% CI, 0.13-0.95; P=0.041)]. Additional analyses of examining malaria-associated inflammatory mediators demonstrated that non-carriers of the CAAGT haplotype had elevated levels circulating IL-1β,P=0.034), IL-6 (P=0.031), and TNF-α (P=0.008).Gene expression analyses revealed reduced levels of HSP70 transcripts in the SMA group (P<0.001) and elevated HSP70 levels in carriers of the CAAGT and CAAAT (P=0.017 and P=0.015) haplotypes. Furthermore, measurement of circulating Hsp70 revealed elevated levels in the SMA group (P=0.062). Taken together, variation in the promoters of HSP70 increases susceptibility to SMA and functionally alters HSP70 gene expression and inflammatory mediator' production.

362

IN VITRO HUMAN CELL FREE EXPRESSION SYSTEM FOR EXPRESSION OF MALARIAL PROTEINS

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Malaria still remains a global concern with no routine vaccines available. Additional vaccine and drug candidate molecules need to be investigated. From previous *Plasmodium yoelii* rhoptry proteome studies, the genes PY01759, PY00763 and a *P. falciparum* PFA0680c were selected. Data from the *Plasmodium* database, PlasmoDB indicates that PY01759 and PY00763 encode proteins of molecular weights 290 kDa and 27 kDa respectively, and are expressed in sporozoite and all erythrocytic stages of *Plasmodium*. In contrast, PF3D7_1361800 the *P. falciparum* orthologue of PY01759 is expressed only in erythrocytic schizonts. Both the orthologs contain Armadillo motifs, which have been shown to play a major role in rhoptry membrane attachment. PFA0680c proteins of molecular weight 25 kDa and the PF3D7_0925900 *falciparum* ortholog of PY00763, encoding a protein of molecular weight 27 kDa are expressed in ring and early trophozoite of the erythrocyte stage. Additional sequence analysis revealed that PY01759 protein is conserved among other apicomplexans. While PY00763 and its *P. falciparum* ortholog PF3D7_0925900 are conserved in *Plasmodium sp.*, PFA0680c is *P. falciparum* specific. In this study, in silico analysis and expression of genes PY00763, PY01759 and PFA0680c was carried out using a new HeLa cell based *in vitro* human cell free expression system. Proteins from the three genes were successfully cloned into plasmid pT7CFE-6his, expressed and purified using Ni-chelating resins. Expressed proteins were identified using rhoptry specific antibodies. The human cell free expression system offers an alternate approach to wheat germ and rabbit reticulocyte lysate cell free expression systems and has the ability to express proteins in three hours.

363

MALARIA PARASITEMIA IN INFANTS RECEIVING IMMUNIZATION IN THE BUEA HEALTH DISTRICT, CAMEROON

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Malaria remains a major cause of mortality and morbidity in infants in sub-Saharan Africa. The intermittent preventive treatment of malaria in infancy (IPTi) has been shown in some settings to be effective in reducing the burden of malaria. However the timing of IPTi in any setting depends on the prevalence and intensity of parasitaemia in infancy. IPTi has not been implemented in Cameroon. However, because contact with health facilities during immunisations represents an opportunity for IPTi, this study sought to determine malaria parasitaemia in infants during immunisation visits in the Buea Health District. A cross-sectional study was conducted in a sample of 220 consenting mother-infant pairs as they came for either the second (DPT2) or third (DPT3) dose of immunisation against Diphteria, Pertusis and Tetanus, or for measles immunisation; administered respectively at 10 weeks, 14 weeks and 9 months of age. A standardised questionnaire was administered to the mothers, after which blood smears collected from the infants were examined for malaria parasitaemia using standard methods. Malaria parasitaemia prevalence at DPT2, DPT3 and measles immunisations were respectively 1.5%, 16.7% and 9.6% (p =0.01). The respective malaria parasite densities were 3.0±25 parasites/µl at DPT2, 16±62 parasites/µl at DPT3 and 3±21 parasites/µl at measles immunisation (p=0.35). No clinical or socio-demographic factors were found to be significantly associated with the presence of parasitaemia. Malaria parasitaemia prevalence was low at DPT2 but relatively high at and after DPT3 suggesting that if IPTi were to be considered in this setting it ought not to start before DPT3. These findings however need to be confirmed in a larger prospective study incorporating an assessment of cost-effectiveness.

364

USING AN ODOR-BAITED DEVICE THAT MIMICS HUMANS TO EXPLORE TECHNICAL OPTIONS AND CHALLENGES FOR ATTRACTING AND KILLING OUTDOOR-BITING MALARIA VECTORS

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Mosquitoes that bite people outdoors can sustain malaria transmission even where effective indoor interventions such as bed nets or indoor residual spraying are already widely used. Outdoor tools may therefore complement current indoor measures and improve control. We developed and evaluated a prototype mosquito control device, the 'Mosquito Landing Box' (MLB), which is baited with human odours and treated with

mosquitocidal agents. We conducted field experiments in Tanzania to assess if wild host-seeking mosquitoes 1) visited the MLBs, 2) stayed long or left shortly after arrival at the device, 3) visited the devices at times when humans were also outdoors, and 4) could be killed by contaminants applied on the devices. Odors suctioned from volunteer-occupied tents were also evaluated as potential low-cost bait, by comparing baited and unbaited MLBs. There were significantly more *Anopheles arabiensis*, An. *funestus*, *Culex* and Mansonia mosquitoes visiting baited MLB than unbaited controls (P≤0.028). Increasing sampling frequency from every 120 min to 60 and 30 min led to an increase in vector catches of up to 3.6 fold (P≤0.002), indicating that many mosquitoes visited the device but left shortly afterwards. Outdoor host-seeking activity of malaria vectors peaked between 7:30 and 10:30pm, and between 4:30 and 6:00am, matching durations when locals were also outdoors. Maximum mortality of mosquitoes visiting MLBs sprayed or painted with formulations of candidate mosquitocidal agent (pirimiphos-methyl) was 51%. Odours from volunteer occupied tents attracted significantly more mosquitoes to MLBs than controls (P<0.001). Odor-baited devices such as the MLBs clearly have potential against outdoor-biting mosquitoes in communities where LLINs are used. Natural human odors suctioned from occupied dwellings could constitute affordable sources of attractants to supplement odour baits for the devices. To curb risk of physiological insecticide resistance the killing agents used should be of different modes of action (other than pyrethroids as used on LLINs).

365

FAUNA INVENTORY AND ASSESSMENT OF *CULICIDAE* NUISANCE IN POST-CONFLICT URBAN AREA: THE CASE OF BOUAKÉ, CÔTE D'IVOIRE

Long Lasting Insecticidal bed Nets **(**LLINs) are one of the most effective and feasible option to control the malaria vectors in endemic zones. Among them, PermaNet®2.0 has proven its efficacy in the field conditions with a remanence of 3 years and a resistance at 20 washes. Data is lacking on assessment of the LLINs efficacy when intensively used in hardship conditions such as in the forest. The aim of the current study was to evaluate in the laboratory conditions the physical status and bio-efficacy of PermaNet®2.0 after 18 months of use in an isolated forest camp in Pokola, Congo. This study was conducted at the CSRS in Côte d'Ivoire on 51 used bed nets originated from Congo. The evaluation included the physical status of the bed-nets that were collected to enumerate the holes and their position, repairs, seam failure, nets size and the assessment of the bio-efficacy according to WHO criteria. The Knock Down (KD) and the mortality rates after 3 minutes exposure of susceptible strains *An. gambiae* Kisumu to mosquito nets were determined respectively after 1 hour and 24 hours post-exposure. Among 51 bed-nets collected, only, 5 nets (9,8%) were in good condition (no hole, white colour). A total of 990 holes were observed corresponding to an average of 19 holes per net. The number of holes was much higher on the big face than on the roof. Furthermore, the majority of holes were found on the border and the lower part of bed-nets, contact points with the bed. There were only 55 repairs counted corresponding to 1,07 repair per net. All the nets were too dirty becoming grey (74,5%) and lost their original color. An increase in nets size was observed with a difference varying between 9 and 26 cm comparing to the original size. Bioassay results indicated a low mortality rate on different sides of the nets to the standard set by WHO (80-100%). However, high knock down rates was found on the roof (98%), 95,3% on the length and 94.0% on the width. The knockdown values complied with WHO criteria (knockdown ≥ 95%). In conclusion, the current study showed that even when PermaNet®2.0 bed nets are torned and are dirty, they still conserve their efficacy even after 18 months in the field.

366

TRANSCRIPTOME PROFILING OF THE PYRETHROID RESISTANT AND SUSCEPTIBLE MOSQUITOES IN THE ASIAN MALARIA VECTOR, *ANOPHELES SINENSIS*

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Anopheles sinensis is one of the major vectors of *Plasmodium vivax* malaria in Asia.Very limited genomic information regarding *An. sinensis* is currently available in public databases, comparing to other malaria vectors in Africa, e.g., *An. sinensis*. RNA-seq provides a fantastic approach to advance the genetics and genomics of *An. sinensis*. To generate novel genomic sequence information for this species and discover transcripts involved in insecticide resistance, we sequenced the transcriptomes of lab colony of *An. sinensis*, as well as those from field pyrethroid resistant and susceptible mosquitoes in China. 454 GS-FLX transcriptome sequencing yielded a total of 624,559 reads (average length of 290bp) using the pool of lab *An. sinensis* colony and field mosquitoes. The de novo assembly generated 33,411 contigs with average length of 493bp. A total of 8057 final ESTs were generated with the Geneontoly (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation. Moreover, 2326 unigens were identified to be the differentially expressed unigenes (DEGs) based on the Reads Per Kilobase per Million mapped reads (RPKM) in field deltmathrin resistant (FR) and susceptible (FS) mosquitoes. There are 261 pathways in DEGs that mapped to the KEGG database, with the predominant unigenes involved in 'Metabolic pathway'. In addition to the P450 Monooxygenases and other metabolic detoxification genes, the distribution of the midgut bacterial and immune genes were also contributed to the DEGs. Furthermore, 2,489 microsatellites were identified and a total of 15,496 sites were estimated to contain Single Nucleotide Polymorphisms (SNPs) and the SNPs of open read frame in 15 contigs were analyzed.The assembled and annotated transcriptome databases provide a significant valuable genomic resource for further study on the important Asian vector, the differential unigenes identified in this study put forward abundant genetic information for further understanding of the molecular mechanism of insecticide resistance. The identified microsatellite and SNP markers will prove useful for extending our current knowledge of the genome organization, for whole genome association studies, and for carrying out comparative genomic analyses within the *Anopheles* mosquito species.

367

A STUDY OF THE BITING PATTERN WITHIN *ANOPHELES GAMBIAE SENSU LATO*

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The recent evidence indicating a modification in the biting pattern of vector populations could pose a challenge to the increased use of Insecticide treated bed nets as a malaria vector control strategy. This modification includes adaptation to biting humans when they are out of bed that is early evening and dawn together with increased outdoor biting. This modification might not necessarily be as a result of an adaptive change in vector behaviour but due to selective pressure against mosquitoes biting when bed nets are in use. This study therefore determined if discrete vector populations with specific biting times exist within *Anopheles gambiae* populations. *An. gambiae* sampled from 6pm to 6am in the field were pooled into four categories based on their time of biting; 6pm-9pm, 9pm-12am, 12am-3am and 3am-6am. Generations were raised on which feeding experiment and selection were performed.

Non anophelines were separated from anophelines and PCR run on *A. gambiae s.l.* for species identification and molecular forms. *Anopheles* sampled comprised of *An. sinensis* (99.3%), *An. funestus* (0.5%) and *An. pharoensis* (0.1%) [n=744]. Different numbers of F1 and F2 progeny were obtained and used for feeding experiments for the different time groups. The percentages for the F1 groups that fed at same time periods as parents in the field (compared with feeding at other time points pooled together) were 90.5%(P=0.013) for 9pm-12am, 66.3%(P=0.029) for 12-3am and 62.3%(P=<0.0001) for 3-6am, whilst F2 had 100%(P=0.228) for 9-12am and 91.2%(P=0.037) for 12-3am. However, irrespective of the period F1 and F2 from different collection times were exposed to feed, a high proportion was found to take a blood meal. PCR run on 134 *An. gambiae s.l.* showed 100% *An gambiae s.s.* Further molecular forms which were determined using restriction enzymes gave 94.8% S forms and 5.2% M forms. Vector behaviours such as host preference, biting pattern and resting have been have been linked to genetic influence but results obtained for F1 and F2 generations showed otherwise probably due to extrinsic factors.

368

BEYOND BUZZING: MOSQUITO WATCHING STIMULATES MALARIA BEDNET USE

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Malaria remains a leading morbidity and mortality cause in Africa. Insecticide treated nets (ITNs) are effective for malaria control. Many organizations have distributed free or highly subsidized ITNs in endemic areas. Nevertheless, some recipients do not use ITNs because of social, environmental or cultural factors. Health education may improve ITN use among people owning but not using an ITN. Here, we hypothesized that watching freshly collected-alive and buzzing-mosquitoes in a household could increase ITN use. To test the hypothesis, we conducted randomized educational intervention in Zomba district, Malawi. Our study site consisted of 285 households and 1199 inhabitants when we began the study. After a series of surveys including bednet distributions, we finally determined that 36 households, which had contained at least one member owing but not using an ITN, were eligible for educational intervention. These 36 households were randomly divided into three educational groups: control (n = 12 households (HHs), 33 people (PP)), education with leaflets (n = 11 HHs, 32 PP), and education with leaflets and fresh mosquitoes (n = 13 HHs, 31 PP). The outcomes were measured by individual ITN uses and household heads' knowledge. The results showed that people who watched freshly collected mosquitoes were about 10 times more likely to use ITNs than those who saw only an educational leaflet or a control group. However, knowledge differences among the groups were not observed. Our results suggest that vector presence realization by direct observation can encourage ITN use and may potentially improve effective ITN coverage for malaria control and elimination.

369

HOW CAN HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM (HDSS) PLATFORM CONTRIBUTE TO MEASURE INSECTICIDE TREATED NETS (ITNS) EFFECTIVENESS IN WEST AFRICAN COUNTRY: EXPERIENCE OF NOUNA HDSS IN RURAL BURKINA FASO?

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Malaria remains the global cause of morbidity and mortality, with most of the burden being in sub-Saharan Africa. Insecticide Treated Nets (ITNs); one of the most effective strategies of Roll Back Malaria is currently rolled out on a large scale. However no much is known about the coverage target and the effectiveness of such a strategy. The aims of the study were to assess the coverage and use of ITNs among pregnant women and under 5 years and its effects on malaria morbidity and mortality in Burkina Faso. A cross-sectional household survey was conducted in the Nouna Health and Demographic Site (NHDSS) in 2011 after the large national free distribution of ITNs. Data were collected from a total of 1050 households, which included 1202 pregnant women and 1114 children using a three-stage cluster sampling procedure in 59 villages of the NHDSS. Overall 97 % of households revealed a possession of at least one ITN in 2011 compared to 89% in 2009. In 2011, 68.6% of children have slept under ITN the last night compared to 25% in 2009. It was 69 % for pregnant women compared to 28% in 2009. The prevalence of clinical malaria among less than five years increased from 20% in 2009 to 21% in 2011. Meanwhile the malaria mortality decreased from 4% in 2009 to 2% in 2011 showing an ITN protective effect (OR=0.66; 95%CI: 0.54-0.80; P<0.05) in target population. In conclusion, although significant progress have been made to improve access of population to ITNs resulting in slight decrease of malaria mortality in NHDSS, still much remains to do for achieving the universal coverage of ITNs needed to attain the MDGs goals . Emphasis should be put on pregnant women and children under five years to ensure their equitable access to malaria prevention materials.

370

ASSOCIATION BETWEEN HOUSE STRUCTURE AND THE INCIDENCE OF MALARIA IN AFRICAN CHILDREN

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Insecticide treated bednets (ITNs) are the most widely adopted intervention for the prevention of malaria in African children. Improved house structure may lower the density of mosquito vectors, offering an additional means of reducing the risk of malaria. This study explored the relationship between materials used in house construction and the incidence of malaria in a cohort of children followed prospectively as part of a randomized clinical trial of antimalarial chemoprevention. A total of 600 children aged 4-5 months living in different houses were enrolled using convenience sampling in Tororo, a rural area with perennial high transmission intensity. Children received an ITN at enrollment and were followed for all their health care needs 7d/wk. Children were randomized to 1 of 4 chemoprevention arms: HIV unexposed infants were randomized at 6 months of age and HIV exposed children at aproximately 6 weeks after cessation of breast-feeding (median age 10 months). Approximately 1 year after the start of the study a survey was performed at each child's house detailing the materials used in house construction. Associations between house structure and the incidence of laboratory confirmed malaria between 6-24 months of age by passive surveillance were estimated using a negative binomial regression model, with measures of association expressed as the protective efficacy (PE=1-incidence rate ratio). The final analyses include 515 children. The prevalence of houses with non-earth floors, non-thatched roofs, and non-mud walls was 14.6%, 43.1%, and 17.5%, respectively. After controlling for chemoprevention, the incidence of malaria was 64% lower in those with a non-earth floor (95% CI, 53-72%, p<0.001), 27% lower with a non-thatched roof (95% CI, 14-39%, p<0.001), and 53% lower with non-mud walls (95% CI, 42-63%, p<0.001). Houses constructed with all non-natural materials were associated with a 70% lower incidence of malaria (95% CI, 60-77%, p<0.001).Most houses in this rural area were constructed with basic materials. Compared to these, houses constructed with materials other than earth, mud, and thatch were associated with a lower incidence of malaria.

371

USE OF INSECTICIDE TREATED NETS AS A CONTROL STRATEGY FOR MALARIA IN WESTERN KENYA

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Globally, 500 million cases of malaria occur annually with an estimated 2.7 million deaths of which 80-90 % are in Sub-Saharan Africa. By 2004, 107 countries were affected and 2b people at risk. In Kenya 30-50% of the out patients are due to malaria, 19% admissions and 42,000 deaths annually. Malaria is an increasing problem among expectant mothers and children under ten and is a major public health problem among the communities within the sugarcane Belt in Kenya. While malaria burden is ever on an increasing, vector control remains a neglected option for malaria control. Intermittent preventive treatment (IPT) with SP drugs has been used in Sub Saharan Africa and is currently the recommended policy in Kenya. However, increasing resistance to SP limits its use for malaria control. Insecticide Treated Nets (ITNs) have been shown to reduce the number of mosquito bites and in turn reduce all cause morbidity and mortality among expectant mothers and children. The objective of the study was to assess the use of ITNs and determine prevalence of malaria and anaemia in expectant mothers attending ANC during high transmission season. To demonstrate the effectiveness of ITNs in community set-ups, 387 expectant mothers attending ANC in Western Kenya located in a malaria endemic area with two high transmission seasons were recruited in. Presence of malaria parasitaemia and hemoglobin concentration, the presence/ absence of fever were determined by measuring temperature of the recruited patients. Of the 387 expectant women recruited, 190(49.1%) of them owned nets, either conventional or long lasting ones. Prevalence of malaria parasitaemia was 28.9%, of which 19.8% (77/387) had malaria (≥ 800 mps/ml of blood). 32.80%, 21.64% and 9.09% of primigravidae, secondigravitae and multigravitae respectively had moderate anaemia (5.0g/dl>7.00g/dl). Parity and net use were significantly associated with malaria status. Occupation was not significantly associated with net ownership and use, pointing to the possible success of awareness campaigns and favorable price affordable to majority. Marital status and education were found to be strong predictors of net ownership and use. It was concluded that net use reduced malaria prevalence during pregnancy.

372

A HIGH-THROUGHPUT LUCIFERASE-BASED ASSAY IDENTIFIES INHIBITORS OF DEVELOPING *PLASMODIUM FALCIPARUM* GAMETOCYTES

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The design of new therapeutic combinations against *Plasmodium falciparum* malaria requires novel drug candidates able to interrupt the disease transmission to the mosquito vector. *P. falciparum* gametocytes, which develop in the human host over a 10 days period, represent the most accessible target stage for such drugs. Considerable effort is currently being taken to identify compounds with late stage gametocytocidal activity, however investigations on the drug sensitivity profile of developing gametocytes, as well as screening methods for early gametocytogenesis inhibitors remain scarce. We developed and optimized a luciferase-based high-throughput screening assay on tightly synchronous early stage *P. falciparum* gametocytes, using a recombinant Pfs16-driven GFP-luciferase NF54 parasite line. The 384-well format assay encompassed gametocyte development from stage I to IIb/III over 72 hours, and was validated by small-scale screening of a collection of 400 compounds with known antimalarial (asexual stage) activity. The assay provided excellent performance with average %CV ≤ 5%, signal to noise ratio above 30 and Z' near 0.8. Only 135 compounds from the collection (1/3 of the total) inhibited early gametocytes at least 50% at 5 µM. The 5 most active hits showed IC50 values around 200 nM. No correlation was found between the gametocytocidal IC50 values of the screening hits and their potency against *P. falciparum* 3D7 asexual stages. Our HTS assay proved reproducible and suitable for the screening of large compound libraries on developing gametocytes. Within the transmission blocking drug discovery strategy, our findings highlight the necessity of screening efforts directed specifically against early gametocytogenesis, and warrant the inclusion of early stage gametocytocidal activity in the desired Target Candidate Profile for antimalarial drug development.

373

USING MALE MOSQUITOES TO CROSS-CONTAMINATE CON-SPECIFIC FEMALES WITH LETHAL DOSES OF PYRIPROXIFEN DURING COPULATION: AN OPTION FOR FUTURE MALARIA CONTROL

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Existing prevention methods such as bednets have significantly reduced malaria cases, but there is consensus that complementary tools are necessary to address ongoing challenges. Studies have shown that *Aedes egypti* mosquitoes, which transmit dengue virus, can pick up lethal doses of pyriproxifen (PPF) during resting and transfer these to their larval breeding sites during oviposition, thus effectively stopping further aquatic mosquito development. Furthermore, it has been demonstrated that males of *Aedes* can contaminate their females during mating. Here, we assessed the possibility of using male *Anopheles arabiensis* to cross-contaminate counterpart females with PPF during copulation, and whether this could reduce fecundity of the females and viability of the resulting eggs. A cup of *An. arabiensis* pupae were put inside a netting cage dusted with 10% PPF, after which 50 emerged males were aspirated into a clean cage in which there were 100 uncontaminated con-specific females of the same age. The mosquitoes were left for 6 days to mate, and then the females were blood fed for 3 consecutive days and provided with egg-laying pots. Relative to a control cage of clean males and females, we assessed number of eggs laid and proportion of the eggs that hatched. Lastly, by dipping the adult males and females into cups with clean 3rd-instar larvae, then assessing larval development to pupation and emergence, we verified whether the males had picked and carried PPF, and determined efficacy of PPF particles potentially picked by the females during copulation. The number of eggs from control cages was significantly higher than from the PPF-dusted cage (P=0.011); the mean number of eggs laid was 12.22 [9.52-14.92] in control and 5.90 [5.35-6.44] in treatment. 37% of the eggs from control hatched compared to 30% in the treatment. Where males from PPF-dusted cage were dipped into larval cups,85% emergence inhibition was observed compared to 6% in control. Similarly, where females mated with the contaminated males were dipped,96% emergence inhibition was seen compared to 1% in control. Pyriproxyfen contaminated males can successful contaminate con-specific females during mating and significantly reduce their fecundity and hatchability rate. With further research, male mosquitoes could potentially be used as a means to disseminate pyriproxyfen in an effort to eliminate malaria though vector population control.

374

DEVELOPMENT OF A GRAVID TRAP FOR COLLECTING MALARIA VECTOR *ANOPHELES GAMBIAE S.L.*

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Effective malaria vector control targeting indoor host-seeking mosquitoes has resulted in a reduction in the number entering houses in many

areas of sub-Saharan Africa, with the proportion of vectors outdoors becoming more important in the transmission of this disease. This study was intended to develop a gravid trap for the out-door collection of the malaria vector *Anopheles gambiae s.l.* based on evaluation and modification of commercially available gravid traps used for culicine collections. Experiments were implemented in an 80 m^2 semi-field system where 200 gravid *An gambiae s.s.* were released each night and replicated for 12 nights. The catching efficacy of the Box, CDC and Frommer updraft gravid traps was compared. Later the Box gravid trap was tested to determine if the presence of the trap over water and the trap's sound affected catch size. Mosquitoes approaching the treatment were evaluated using electrocuting nets or detergents added to the water in the trap. Based on the results of these experiments, a new gravid trap that provided an open, unobstructed oviposition site was developed and evaluated. Box and CDC gravid traps collected similar numbers of mosquitoes (Odds ratio (OR) 0.8, 95% confidence interval (CI) 0.6-1.2; $p = 0.284$), whereas the Frommer updraft gravid trap caught 70% fewer mosquitoes than both traps (OR 0.3, 95% CI 0.2-0.5; $p < 0.001$). The number of gravid females approaching the Box trap was significantly reduced when the trap was positioned over a water-filled basin compared to a small pond (OR 0.7 95% CI 0.6 - 0.7; $p < 0.001$). This effect was not due to the sound of the trap. Catch size increased by 60% (OR 1.6, 1.2 - 2.2; $p = 0.001$) with the new trap. In conclusion, gravid *An. gambiae s.s.* females were visually deterred by the presence of the trapping device directly over the oviposition medium. Based on these investigations, an effective suction gravid trap was developed that provides open landing space for egg-laying *Anopheles* mosquitoes.

375

RAPID INCREASE IN MALARIA SERVICES FOR PREGNANT WOMEN IN SOUTH SUDAN

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Administration of two doses of intermittent preventive treatment (IPT) with sulfadoxine/pyramethamin (SP) and consistent use of LLINs reduces pregnancy complications and neonatal deaths from malaria. In 2010, a household survey in South Sudan showed that only 22.7% of pregnant women received two doses of SP. The second phase of the Sudan Health Transformation Project (SHTP II) focused on prevention of malaria in pregnancy. By outreach to pregnant women to increase attendance at ANC services, training of maternal health provider staff in IPT, and provision of medications and commodities with minimal stockouts, the SHTP II aimed to increase the number of pregnant women who received IPT 2. In addition, distribution of LLINs during ANC and under 5 services increased sleeping under an LLIN. During the three years of the SHTP II, IPT2 visits increased 229% and coverage of IPT 2 increased from from 29% to to 53%. ANC 1 increased from a basleine of 53% to 83% of PW, and ANC 4 increased from 23% to 49% of PW. In conclusion, significant increases in utilization of ANC 1 And ANC 4 services improved both IPT 2 and LLIN distribution and use, contributing to overall improvements in maternal and newborn care. Increasing access to malaria in pregnancy prevention efforts by integrating community awarenes, outreach and improved service delivery is possible even in the challenging fragile state of South Sudan.

376

TARGET SITE AND METABOLIC MECHANISMS CAUSE EXTREME INSECTICIDE RESISTANCE AND CROSS-RESISTANCE IN *ANOPHELES GAMBIAE S.S.* FROM WEST AFRICA

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Malaria control depends on mosquito susceptibility to insecticides. With resistance to pyrethroids and DDT now widespread, carbamates are an increasingly important alternative for indoor residual spraying (IRS). Yet mechanisms of resistance to carbamates are poorly understood and critical knowledge of potential cross resistance with other insecticide classes is lacking. We assayed insecticide resistance in *Anopheles gambiae* mosquitoes from southern Côte d'Ivoire and applied synergist, target site assays and whole genome microarrays to investigate resistance mechanisms. Mosquitoes were resistant to insecticides from all four approved classes. Such complete resistance, which includes exceptionally strong phenotypes, presents a major threat to malaria control. The G119S target site resistance mutation was strongly associated with bendiocarb survivorship, but bioassays with PBO, which synergises P450 enzymes, restored significant insecticide efficacy, suggesting a previously unappreciated role of metabolic resistance in carbamate resistance. This observation was confirmed by microarray analyses, which implicated the involvement of multiple P450s in carbamate resistance. The role of the strongest candidate P450, Cyp6M2, which is also linked with pyrethroid and DDT resistance in *An. gambiae* was validated via production of a bendiocarb resistant phenotype in transgenic Drosophila melanogaster. Our results demonstrate strong roles for target site and metabolic mechanisms in producing extreme levels of carbamate resistance in *An. gambiae*, in addition to a concerning potential for cross resistance via overexpression of a specific P450 gene.

377

HOUSE DESIGN MODIFICATIONS FOR "MOSQUITO-FREE HOMES": AN INNOVATIVE, EFFECTIVE AND ENVIRONMENTALLY SOUND ALTERNATIVE TO CHEMICAL USE

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Mosquito-proofing homes have been a fundamental technique for malaria control since early 1900s. Simple non-chemical modifications of a typical rural house design using ventilated ceilings mats can be an effective, acceptable and relatively inexpensive method of reducing entry of indoor mosquito vector densities and consequently decreasing malaria transmission. Ten treatment houses were modified with ventilated ceilings of papyrus mats and insecticide treated netting and tested against ten control houses. To determine densities of mosquitoes resting in homes the pyrethrum spray method was used to simultaneously collect indoor resting malaria vectors in intervention and control houses. Each house was sampled a total of 8 times for 4 months, resulting in a total of 80 sampling efforts for each treatment. Community response was investigated by a questionnaire survey. House modification reduced malaria vectors house entry by 78-86% compared to unmodified houses. Geometric mean density of *An. gambiae s.l.* and An. *funestus* in modified houses were significantly lower ($t158=6.35$, $P<0.0000$ and $t158=2.79$, $P=0.006$, respectively) compared to controls. There was a 84% (OR 0.16, 95% CI 0.07-0.39, $P=0.0000$) and 87% (OR 0.13, 95% CI 0.03-0.5, $P=0.0004$) reduction in the odds of *An. gambiae s.l.* and An. *funestus* presence in modified houses, respectively, compared with unmodified houses. A survey in 270 households indicated that 99% of the respondents had willingness and ready to modify their houses for malaria control. Other reasons

associated with the modification that were cited by respondents to favour the strategy were temperature regulation (50%) and house beautification (33%). Cost was cited as the main challenge where 26% against 68% said was affordable. House modifications involving ventilated ceilings have the potential to reduce human exposure to malaria vectors, and thus parasite infection in malaria endemic regions and likewise reduce the use of chemicals in the environment. Ceilings made from locally available materials are likely to be well accepted.

378

HOW THE MALARIA VECTORS ADAPT TO THE USE OF INSECTICIDE-TREATED NETS BY AFRICAN POPULATIONS?

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Malaria remains one of the main health scourges affecting Africa. The fight against this disease is based on vector controls that rely primarily on indoor residual spraying and the use of long lasting treated bed nets (LLINs). However the effectiveness of these tools now faces the emergence of insecticide resistance and adaptation. This study evaluates the consequences of bed nets use on vectors resistance to insecticides, their feeding behavior in malaria transmission in a rural Senegalese village, Dielmo after deployment of LLINs in July 2008. Adult mosquitoes were collected by human landing catches (HLC) monthly from January 2006 to March 2013 and by pyrethrum spray catch (PSC). Anopheline identification was performed by loupe and sub-species by PCR. The presence of circumsporozoite protein (CSP) of Pl. *falciparum* and the blood meal origin was detected by ELISA, and kdr mutations were investigated by PCR. We demonstrated that, after bed nets implementation, insecticide susceptible mosquitoes (wild kdr genotype) had a reduced lifespan, but they rapidly adapted their feeding behavior, becoming more exophageous and zoophilic, and biting earlier during the night. In the meantime, insecticide-resistant specimens (kdr L1014F genotype) increased in frequency in the population, with an unchanged lifespan and feeding behavior. Better, mosquitoes collected in February-March 2013 between 7pm to 11am by HLC showed that over 65% of anophelines were caught in daylight (07am and 11am). These disturbing results show anopheles adaptative capacity to circumvent strategies aimed at reducing malaria transmission. Due to their extraordinary adaptative skills, *Anopheles* mosquitoes continue to be excellent malaria vectors even after mass deployment of insecticide treated bed nets. Thus, by using nets to protect us, are we not going to provide the *Anopheles* with the entire "arsenal" needed to hit much harder?

379

LABORATORY EFFICACY OF THE PYRROLE INSECTICIDE, CHLORFENAPYR ALONE OR IN COMBINATION WITH PYRETHROID FOR CONTROL OF PYRETHROID SUSCEPTIBLE AND RESISTANT MOSQUITOES

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Pyrethroid resistant mosquitoes are becoming increasingly common in parts of Africa. There is an urgent need to develop alternative insecticides for use in indoor residual spraying (IRS) to supplement the pyrethroids for malaria control. Because there is limited number of new public health insecticides coming onto the market since the event of pyrethroids, certain compounds from the agricultural portfolio of insecticides such as the pyrrole chlorfenapyr have already shown great potential for control of pyrethroid resistant mosquitoes. I furthered on to explore under laboratory conditions the potential of putative dosages of a wetable powder (WP) formulation of chlorfenapyr for IRS against anopheles and the possibility of combining such formulation with the pyrethroid alphacypermethrin for IRS to control both resistant and susceptible mosquitoes, on wooden and cement substrates. Chlorfenapyr WP range 200-500mg/m2 was very effective against anopheles ($P<0.05$) but activity on cement fell short just after 1 month. The combination of chlorfenapyr/alphacypermethrin was also very effective and controlled both resistant and susceptible mosquitoes. Further studies would be needed in experimental huts to estimate the mass killing impact of chlorfenapyr WP alone and its combination with pyrethroid. More advanced technology such as micro encapsulation is needed to enhance the residual life of this useful insecticide on concrete.

380

AN ANALYSIS OF THE COST OF FITNESS OF KDR INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* MALARIA VECTORS

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Previous studies have shown that insects suffer a fitness cost when they try to resist insecticides in their environment. The rationale for this work is to determine if the fitness cost will have a negative impact on wild resistant *Anopheles gambiae s.s.* To achieve this, 100 bloodfed adult female mosquitoes were collected from two villages, Okyereko (resistant) and Dodowa (susceptible) and given optimal conditions to lay eggs which were bred to adulthood and fitness parameters determined. Results obtained indicated that adult survival were 20 and 40 for Okyereko and Dodowa respectively. Analysis from binary logistic regression reveals that average egg laid were higher for kdr negative (178,139) than for kdr positive (100,127) for both sites Okyereko and Dodowa respectively. Similarly, fecundity (139,44) and growth in terms of productivity were both higher for *kdr* - than for *kdr* + however, egg retention was lower for *kdr* - (39,44) than for *kdr* + *An. gambiae* (100,37).Average survival days was also higher for *kdr* - (6,7) than for *kdr*+ (0,3), wheras longevity was (21,16) and (0,10) respectively for *kdr*- and *kdr*+ mosquitoes. A fitness cost was found between *kdr* and longevity as above binary analysis proved longevity of the *An. gambiae* mosquitoes to be significant $P\leq0.05$.This finding of a fitness cost with respect to longevity may prove useful in reducing ability of the malaria vector to transmit malaria parasites.

381

FUNGAL APPLICATION ON NETTING SUBSTRATE FOR MALARIA VECTOR CONTROL IN GHANA

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Recent malaria control strategies, which partly involve the use of fast killing chemical agents to prevent infectious mosquito bites, are being threatened globally by the gradual development of mosquito resistance due to selection pressure. Fungal entomopathogens show.potential as an alternative biological control agent against anophelines. Like conventional insecticides, fungal spores act via contact, typically killing the mosquito in 4-10days, depending on exposure dose, viability, and virulence of the fungal strain. This study aims at determining if the fungus, Beauveria bassiana, can be delivered to mosquitoes on netting materials which could be used in households in Ghana. Anopheline larvae will be collected over a period from three select communities in southern Ghana and raised in the insectary. A minimum of twenty-five (25) each of cotton and polyester bed nets will be treated with spores of B. bassiana suspended in Shellsol solvent by dipping. Three portions of the individual nets will be cut out (30x30cm) at randomly selected positions and stored in aluminum foils for cone bio-assays. The first generation (F1) adults from the larval collection will be used for WHO cone bio-assays on the netting materials cut out

earlier, with controls setup, and observed over a period of sixteen (16) days after exposure. These cut out net pieces will be stored in optimal conditions for the tests to be repeated every week to determine the rate of decay of the spores as well as the longevity of their action. Results are expected to exploit the possibility that bed nets treated by dipping in a suspension of fungal spores dissolved in Shellsol would be a promising alternative for field implementation in Ghana. Biological control with fungus-impregnated netting materials could provide a means to target host-seeking mosquitoes upon house entry, and has potential for use in integrated vector management strategies, in combination with chemical vector control measures, to supplement malaria control in areas with high levels of insecticide resistance.

382

EXPERIENCES OF BENIN REGARDING INDOOR RESIDUAL SPRAYING (IRS) IMPLEMENTATION: PERFORMANCES AND LIMITS

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The National Malaria Control Program (NMCP) has been implementing a vector control strategy based on Indoor Residual Spraying (IRS), using bendiocarb insecticide, in Benin since 2008. As a matter of fact, Benin decided to use IRS especially in order to reinforce the action of the Long Lasting Insecticidal Nets (LLINs). From 2008 to 2010 and 2011 to 2012, IRS was respectively implemented in the Southern Benin, precisely in Oueme department, and in the North, precisely in Atacora department. These 2 trials were carried out at large scale, covering more than 500,000 inhabitants but in a context of high resistance of *Anopheles gambiae* to pyrethroids. And, that is why the IRS strategy adopted was based on the use of a non-pyrethroid, the bendiocarb insecticide. The application dose was 0,4g/m^2 of bendiocarb on the walls. Indeed, applications were undertaken by volunteers selected in the community and the coverage rate was more than 85%. To perform any spraying, a manual atomizer with previous pressure HUDSON XPERT type was used by the sprayers who, prior to any application, were protected with a lab coat, gloves, boots and a helmet for their own security. Entomological parameters registered in the control areas were then compared to those of intervention sites. The results obtained were actually encouraging. In southern Benin, the study has shown a drastic decrease in *An. gambiae* biting rate in areas under IRS intervention. Besides, ELISA tests were negative for circumsporozoite (CS) antigen of *Plasmodium falciparum* during the whole period of intervention. Moreover, nobody received infected bites (EIR = 0 from January to July). Similar results were recorded in the North, in Atacora department, with more than 70% malaria transmission reduction. In Benin, bendiocarb insecticide was found to be a good alternative for IRS strategy in areas where *An. gambiae* has developed a high resistance to pyrethroids. However, after 2 years of IRS in the North, bendiocarb resistance in *An. gambiae* due to the use of high quantity of various insecticides by farmers against cotton pests was registered. Therefore, facing the emergence of bendiocarb resistance, the NMCP decided to replace this insecticide by pirimiphos methyl (OP) in 2013. We wonder what will become of IRS in Benin after pirimiphos methyl resistance. Indeed, the resistance has been noted for all types of insecticide.

383

AGE-DEPENDENT SUSCEPTIBILITY OF MOSQUITOES TO IVERMECTIN

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Ivermectin administration to humans can kill mosquitoes and may impact malaria transmission, as reported previously. Several other studies have demonstrated that ivermectin affects *Anopheles gambiae* survivorship, delays re-feeding rates, increases knockdown, delays recovery, reduces fecundity, and multiple blood meals with ivermectin compound mosquito mortality. As reported, susceptibility of mosquito vectors to insecticides has been demonstrated to be age-dependent and upon ingestion of such insecticides, several detoxification mechanisms could be induced more or less efficiently depending on mosquito age. We are investigating the age dependent susceptibility to ivermectin in *Anopheles gambiae* in order to evaluate ivermectin mass drug administration as a new and integrated malaria control tool. Our hypothesis is that ivermectin may induce overexpression of some detoxification genes to eliminate the compound before it reaches its target. and the extent of this induction may be age dependent. We performed blood-feeding experiments with ivermectin on 2, 6 and 14 days post emergence. Preliminary results support the hypothesis of age-dependent toxicity of ivermectin to mosquitoes. Planned experiments will measure the activity of three types of metabolic enzymes involved in xenobiotic detoxification (carboxylesterase, glutathione-S-transferase and P450-monooxygenase) to determine their impact on ivermectin tolerance and on age-dependent susceptibility. We will also perform transcriptomic analysis using RNA-seq to determine the genes induced by ivermectin with special emphasis on detoxifying genes, and to compare the age effect on the expression of these genes. We expect these data will identify potential metabolic resistance mechanisms to ivermectin in malaria vectors. Such data would help to evaluate the strategy of using ivermectin as a systemic drug to control malaria transmission.

384

INVESTIGATION OF DETOXIFICATION GENE PROFILES IN DELTAMETHRIN RESISTANT *AEDES AEGYPTI* POPULATIONS FROM DISTINCT GEOGRAPHICAL ORIGIN

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Aedes aegypti is a cosmopolite mosquito, vector of arbovirosis. The worldwide studies of its insecticide resistance have demonstrated a strong loss of susceptibility to pyrethroids, the major class of insecticide used for vector control. French oversea territories such as French Guiana (South America), Martinique and Guadeloupe islands (Lesser Antilles) as well as New Caledonia (Pacific Ocean), have encountered such resistance. In 2009, we initiated a research program on the pyrethroid resistance in French Guiana, Guadeloupe and New Caledonia. *Ae. aegypti* populations were tested for their deltamethrin resistance level then screened by an improved microarray developed to specifically study metabolic resistance mechanisms. Cytochrome P450 genes were implicated in conferring resistance. CYP6BB2, CYP6M11, CYP6N12, CYP9J9, CYP9J10 and CCE3 genes were upregulated in the studied populations and were common to other populations at a regional scale. The implication of those genes in resistance phenomenon is then strongly suggested. Other detoxifying genes were also upregulated at lower scale. These first results were complemented by screening for target site mutations on the sodium channel voltage dependent gene (eg. kdr). The V1014I mutation on the DIIS6 was observed in the population from French Guiana and Guadeloupe. After establishing resistance backgrounds we will undertake further research on the relationship between the environment and the profile of resistance. Ecological and anthropogenic observations, results of field and lab experimentations, and vector control practices will be related to mosquito genomic and transcriptomic profiles that will help us to better understand the dynamics of resistance.

385

THE DURABILITY OF LONG-LASTING INSECTICIDE-TREATED NETS IN ZAMBIA - BASELINE RESULTS FROM A TWO-YEAR PROSPECTIVE COHORT STUDY

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Long-lasting insecticide treated nets (LLIN) are a key malaria intervention in sub-Saharan Africa. Estimating net longevity and need for replacement is critical for long-term malaria control. Manufacturers report that LLINs last 3-5 years. However, an unpublished study in Zambia suggests that LLINs physically deteriorate before 27 months. In 2012, the President's Malaria Initiative (PMI) collaborated with the United States Peace Corps on an ongoing net durability study in Zambia. With assistance from the National Malaria Control Center, staff from the PMI and the Peace Corps Malaria Coordinator trained Peace Corps Volunteers and local Zambian counterparts in interviewing and data collection. The study examines physical integrity and insecticide persistence in LLINs over time. LLINs distributed in two different provinces, Permanets® (Luapula Province) and Olysets® (Northern Province), are followed every 6 months to count and measure holes (size approximated by thumb, fist, and head) for 2 years. Households are surveyed about net care and usage. We present information on the physical durability of 12-month-old nets. We enrolled 999 LLINs; 505 Permanets® and 494 Olysets®. Of the 999 households, 91.9% of those interviewed used the study netthe night before the interview. 71.0% of nets were previously washed, and Permanets® were slightly more likely than Olysets® [RR 1.08 (1.02-1.15)] to have been washed. LLINs were hung most commonly over reed mats (24.3%); households in Luapula Province were less likely to use reed mats [RR 0.73, CI (0.56-0.95)]. About one-third of the nets had holes of any size (n=307, 30.7%), with no difference between type of net; 34.4% of Permanets® vs 34.8% of Olysets® [RR 0.97, CI (0.89-1.05)]. The proportions of the size of holes did not differ between types of nets. Nets hung over reed mats were more likely to have holes [RR = 1.37, CI (1.06-1.78)]. In sum, nets are not as durable as expected, especially when hung over reed mats. A strong collaboration between the Peace Corps and the PMI makes this village-based study possible.

386

LARVICIDAL ACTIVITY OF A PHOTO-ACTIVATED PORPHYRIN AND NEEM PORTION FORMULATIONS AGAINST *ANOPHELES GAMBIAE* S.L

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The spread of insecticide resistance among *Anopheles* mosquitoes raise the needs for new insecticidal malaria vector control strategies. Photo-activated porphyrins induce lethal tissue damage to organisms through oxidative stress mechanisms. Neem extracts are known to possess insect growth regulator properties on mosquito larvae. Thus, portions of neem (*Azadirachta indica*) plant could constitute candidate carriers for the meso-substituted cationic porphyrin (C12) against *Anopheles* mosquitoes. Based on this hypothesis, the larvicidal efficacy and the delayed effect of combinations of the two types of larvicidal tools were assessed on larvae of wild-caught *An. gambiae s.l.* from Burkina Faso. In outdoor trays' experiments, the C12 formulations were assayed against batches of 60 larvae treated in water samples from potential *Anopheles* larval breeding sites. Among the tested porphyrin neem formulations for efficacy, the porphyrin neem fruit combination (NF-C12) was able to induce about 82.7±6.3% mortality after 44h of exposure irrespective of breeding site water type of treatment. However, while favoring a rapid killing effect against mosquito larvae, the combination porphyrin neem reduced by about half the delayed effect of neem portion against *An. gambiae s.l.* larvae. In the contexts of management of integrated vector control and resistance management, the neem fruit used as C12 porphyrin carrier may constitute a promise larvicidal tool against *An. gambiae s.l.*.

387

CHARACTERIZATION OF BACTERIAL DIVERSITY IN THE MIDGUTS OF WILD *ANOPHELES GAMBIAE* MOSQUITOES AND THE IMPACT OF NATURAL MIDGUT BACTERIAL COMMUNITIES ON *PLASMODIUM FALCIPARUM* SPOROGONIC DEVELOPMENT

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Malaria remains the most impactful vector-borne disease worldwide and a number of methods used to control the disease are focus on combined interventions, among them the insecticide-treated nets (ITNs) and treatment with effective antimalarial drugs. Despite these methods some resistance have been observed and due to unavailability of an effective vaccine, a recent studies now focus on tripartite interactions between vectors, parasite and the vector's intestinal microflora at the molecular level have revealed complexities that can drastically affect immune responses and *Plasmodium* densities in mosquitoes. Now the influence of environmental factors on the *Plasmodium* transmission success have been study, we present here the diversity of natural migdut bacteria at different stage of *Anopheles gambiae* population in four localities in Yaounde Cameroon. Bacterial communities of wild *An. gambiae* mosquitoes was recovered using a conventional culture technique on MacConkey medium and sequencing using the 16S rRNA gene. Interestingly, the results gut community revealed that the enterobacteriaceae family was dominant in all developmental stage and the main genera were Escherichia-shigella and Serratia with 55% and 38% respectively in adult female mosquitoes. This diversity was previously described except here where we report the presence of *Delftia* genus in *Anopheles* mosquito; the genus Enterobacter was found at larval stage and adult males and this is in contrast with others studies. We next measured the effects of these natural bacterial isolates to *Plasmodium falciparum* infection prevalence and intensity over multiple infectious feedings using a meta-analysis. Our investigations to verify the potential role of field isolated bacteria shown that the prevalence and intensity of *Plasmodium falciparum* infection was drastically reduced when mosquitoes were first challenged with *Pseudomonas stutzeri, Serratia marcescens* and *Escherichia coli* whereas *Enterobacter* sp has no detectable effect which is contrast with the recent study. The details of natural mosquito gut and their effects on natural *Plasmodium falciparum* are now study but the mechanisms used by bacteria remain poorly elucidated. Deciphering microbe-pathogen interactions remains the challenge and may offers new perspectives to control malaria transmission.

388

BITING PATTERNS AND SEASONALITY OF *ANOPHELES GAMBIAE SENSU LATO* AND *ANOPHELES FUNESTUS* GROUP UNDER PROLONGED USE OF INSECTICIDE-TREATED BED NETS IN KAMULI DISTRICT, UGANDA

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We investigated the biting patterns and seasonal abundances of *Anopheles gambiae s.l.* and *An. funestus* mosquitoes under prolonged use of insecticide-treated bed nets (ITNs) in Kamuli District, Uganda. The number of mosquitoes caught biting humans, the indoor and outdoor human biting densities and rates during different hours of the night, and mosquito abundances for the twelve-month sampling period in both intervention and non-intervention zones are being reported. Hourly indoor and outdoor catches of human biting mosquitoes were sampled from 19.00 to 07.00 hours for four consecutive nights each month using bed net traps in forty-eight houses randomly selected from five intervention villages and five non-intervention villages. The indoor and outdoor human-biting fractions, time of biting of the anophelines and climatic data were recorded from January to December 2010. Approximately four times more *Anopheles* mosquitoes were caught biting humans in the non-intervention zone than in the intervention zone, with *An. gambiae s. l.* catches exceeding those of *An. funestus*. In both zones, peak night biting occurred between 23.00 and 05.00 hours, indicating that ITNs should be effective. The majority of bites occurred between 03.00 and 06.00 hours for both *An. gambiae s. l.* and *funestus* group. Outdoor biting densities of *An. gambiae s. l.* exceeded the indoor biting densities throughout night in both zones, while the indoor and outdoor human biting densities of *An. funestus* group were apparently equal. The outdoor and indoor human biting rates were similar in both zones. In the intervention zone, the abundance of *An. gambiae s.l.* was rainfall-dependent, while the *An. funestus* group could shrive with or without rain fall. In the non-intervention zone, both *An.gambiae s.l.* and *An.funestus* mosquitoes shrived all year round regardless of the amount of rainfall. Considering the effectiveness of ITNs and biting patterns and seasonal abundances exhibited by *An. gambiae s.l.* and *An. funestus* mosquitoes in Kamuli district, scaling up the use of Long Lasting ITNs (LLINs) in combination with indoor residual spraying, environmental management and improved house designs in the context of integrated vector management may be the appropriate vector control strategy.

389

MODULATION OF *ANOPHELES* GENE EXPRESSION BY NITROQUINE

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Antimalarial drugs may impact mosquito's defense against *Plasmodium* parasites. Our previous study showed nitroquine significantly reduced infection of *Anopheles stephensi* by *P. yoelii*, but the underlying mechanism remains unclear. In order to understand how transmission capacity of *An. stephensi* was affected by nitroquine, we explored the transcriptome of adult females after different treatments and examined changes in gene expression profiles. As the *An. stephensi* genome has not yet been published, we adopted a new method to identify process-related genes based on their differential expression.We extended massively parallel sequencing and data analysis (including gene discovery, expression profiling, and function prediction) to *An. stephensi* before and after *Plasmodium* infection with or without nitroquine treatment. Using numbers of reads assembled into specific contigs to calculate relative abundances (RAs), we categorized the assembled contigs into four groups according to the differences in RA values: infection induced, infection suppressed, drug induced, and drug suppressed. We found both nitroquine in the blood meal and *Plasmodium* infection altered transcription of mosquito genes implicated in diverse processes, such as immunity (*e.g.*, pattern recognition receptors, extracellular signal modulators, and intracellular signal transducers), cytoskeleton, adhesion, and oxidative stress. The differential gene expression may have promoted defense responses of *An. stephensi* against the parasite and thus decrease its infectivity. Our study indicated that nitroquine may regulate several immune mechanisms in the mosquito against *Plasmodium* at the level of gene transcription. This highlights the need for better understanding of antimalarial drug's impact on parasite survival and transmission. In addition, our data largely enriched the existing sequence information of *An. stephensi*, an epidemiologically important vector species.

390

HUMAN ANTIBODY RESPONSE TO *AEDES AEGYPTI* SALIVARY PEPTIDE AS A NEW INDICATOR FOR ASSESSING THE RISK OF ARBOVIRUSES TRANSMISSION AND THE EFFICACY OF VECTOR CONTROL, IN DENGUE AND CHIKUNGUNYA TRANSMISSION AREAS

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New indicators are urgently needed to assess the human exposure to *Aedes* mosquito bites and therefore, to evaluate the risk of dengue (DENV) and chikungunya virus transmission, and the efficacy of vector control strategies. Previous studies have demonstrated that human antibody (Ab) responses to *Aedes aegypti* Nterm-34kDa salivary peptide could represent promising tool for evaluating the human-*Aedes* contact. In order to validate this indicator, we investigated its usefulness for measuring both, the risk of arboviruses transmission and the efficacy of vector control. Specific IgG response to Nterm-34kDa peptide was evaluated from 208 individuals, within a cross-sectional study conducted in urban area of Vientiane city in Laos, Southeast Asia. The specific IgG responses in individuals living in low urbanized neighbourhoods were higher than those from the high urbanized ones ($P < 0.0001$). A similar pattern was observed concerning the prevalence of recent DENV infection. This suggests that the exposure of vectors bites, and therefore the risk of DENV transmission, is probably higher in low urbanized areas than in the more urbanized ones. Human IgG response to Nterm-34kDa peptide was also assessed from 102 individuals living in urban area of Saint-Denis in La Reunion Island, Indian Ocean, before and after the implementation of vector control against *Aedes* mosquito. Specific IgG response decreased just since 2 weeks ($P < 0.0001$), and until 4 weeks post-intervention ($P = 0.0002$). This decrease appeared, during the first week post-intervention, to be associated to the decline of *Aedes* mosquito density, estimated by entomological parameters. This indicates a probable earlier but not longer reduction of exposure to *Aedes* bites after vector control implementation. Altogether, these results showed that human IgG Ab response to *Aedes aegypti* Nterm-34kDa salivary peptide could be a pertinent indicator for, i) predicting areas with higher risk of arboviruses transmission and, ii) evaluating the efficacy of vector control programs.

391

MOSQUITO AND FLAVIVIRUS SURVEILLANCE FROM FLOOD AFFECTED AREAS IN THAILAND

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Over 10% of arable land was destroyed and 815 deaths resulted from the disastrous flooding of 2011 in Thailand. Water levels reached waist height in some areas of Bangkok by late October and remained elevated well into January 2012. The threat of vector-borne disease, such as Japanese encephalitis or dengue, is always a concern in high-density populations and Bangkok is no exception. We surveyed mosquito populations using CO_2 - baited, CDC light traps in 7 flood affected areas in Bangkok and nearby Pathumthani province during and immediately after the flooding. Our goal was to identify community composition, abundance, and the presence of flavivirus in collected mosquitoes. A total of 110,510 and 124,588 mosquitoes were trapped during and after the flood, respectively. Our sample consisted of 8 genera: *Culex sp., Mansonia sp., Aedes sp., Anopheles sp., Lutzia sp., Armigeres sp., Coquillettidia sp.* and *Uranotaenia* species. A total of 18 species were captured during the flood and 22 species were collected after flood waters had begun receding. *Culex* mosquitoes, the most abundant specimens, were pooled by species and location and randomly selected for flavivirus RNA screening using SYBR Green I-based real-time RT-PCR assay. All 217 tested pools (6,905 mosquitoes) of *Cx. tritaeniorhynchus, Cx. vishnui,* and *Cx. gelidus* were negative for flavivirus. This study provides a better understanding of mosquito diversity, abundant, and mosquito-transmitted flavivirus situation from flood affected areas in Thailand.

392

GENETIC BASIS OF VECTOR COMPETENCE FOR FIELD DENGUE VIRUS ISOLATES IN A NATURAL POPULATION OF *AEDES AEGYPTI*

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Successful infection and dissemination in the mosquito vector are prerequisites for dengue virus (DENV) transmission among humans. The ability of a mosquito to become infected following ingestion of infectious blood and to subsequently develop a disseminated infection varies substantially between and within populations of the primary DENV vector, *Aedes aegypti*. Knowledge of the genetic basis of *Ae. aegypti* vector competence for DENV mainly derives from laboratory-tractable systems that consist of artificially selected mosquito lines and a single reference DENV strain. DENV exists in nature as four antigenically distinct serotypes with considerable intra-serotype genetic diversity. We characterized the genetic basis of vector competence for different DENV isolates from two serotypes in a natural *Ae. aegypti* population. Mosquito isofemale families were derived from wild eggs collected in Kamphaeng Phet, Thailand. Families were experimentally exposed to low-passage DENV isolates obtained from the serum of human patients. We used a quantitative genetic approach to survey genetic factors controlling vector competence based on both inter- and intra-family variation. Analysis of inter-family variation revealed that mosquito genetic factors strongly influence natural vector competence in this *Ae. aegypti* population. Analysis of intra-family variation allowed us to locate some of these factors in the *Ae. aegypti* genome by genetic mapping. Importantly, whereas some mosquito genetic factors had a generalist effect across DENV serotypes and isolates, others acted in a virus isolate-specific manner. Thus, multiple genetic factors control vector competence for DENV in this natural *Ae. aegypti* population but the effect of these factors is modulated by the viral genome. Our results demonstrate that DENV transmission is controlled by a complex interaction between the mosquito and viral genomes. They emphasize the importance of taking into account viral genetic diversity for understanding the genetic basis of mosquito vector competence in natural populations.

393

ROLE OF NUTRITIONAL RESERVES AND BODY SIZE IN *ANOPHELES GAMBIAE* MALES MATING SUCCESS

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A better knowledge of the different parameters that account for male mating success in the wild is critical to the development of genetic control strategies. In this study, we measured energy budgets (total sugar and glycogen) as the daily energetic investment in swarming males of *Anopheles gambiae s.s.* M and S molecular forms from two different field locations, VK7 and Soumousso. We also looked at the difference between energetic reserves in mated males compared to unmated ones, and assessed wing length in both molecular forms to explore whether this phenotypic trait was involved in swarming behavior or mating success. The current study showed that the energetic cost of 25 minutes of swarming was around 50% of the male's sugar (M form: 48.54%, S form: 56.27%) and glycogen (M form: 53.13%, S form: 59.04%) reserves. However, no difference in carbohydrate content was observed between mated and unmated males. Mated males were found to be bigger than unmated ones, while intermediate size of males is advantageous in mating system, both in M and S molecular forms and when collected in two different locations. Regardless of the collection location, no difference in wing size was observed in swarming males collected early or late during a particular swarm. The results are discussed in the context of ecological and sexual selection.

394

ASSESSING TRANSMISSION OF LYMPHATIC FILARIASIS IN ENDEMIC COMMUNITIES WITH AT LEAST FIVE ROUNDS OF MASS DRUG ADMINISTRATION IN GHANA

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There has been a critical question of when to stop Mass Drug Administration (MDA) in lymphatic filariasis (LF) endemic communities. After at least five (5) rounds of MDA with ivermectin and albendazole, it is expected that microfilaraemia should reach a minimum threshold in the human population such that the vectors (predominantly *Anopheles gambiae*) may not be able to pick up microfilaria during a blood meal. In Ghana, studies have indicated that some vectors particularly An. melas and Mansonia spp could transmit at low levels of microfilaraemia thus keeping a residual transmission of the disease even after more than seven rounds of LF MDA. Using Pyrethrum Spray Catches (PSC), mosquitoes were sampled from Ayensuako, Gyaahadze, and Mankrong communities where more than 5 rounds of MDA have been done. All mosquitoes caught were morphologically identified and dissected for infection with W. *bancrofti* and the cibarial armature examined for the various species. A total of 550 mosquitoes were collected. Distribution of mosquitoes in these endemic communities was predominantly *An. gambiae s.l.* - 462/550 (84.0%). The proportion of other mosquitoes species were An. *funestus* 9/550 (1.6%), An. pharoensis 1/550 (0.2%), *Culex* sp 57/550 (10.4%). and Mansonia *sp.*, 21/550 (3.8%). For all samples, microscopy was negative for LF parasites. In general, the cibarial teeth of the *Anopheles* species were significantly more than those observed for *Mansonia sp.* and for *Culex sp.* Although

the numbers of mosquitoes collected were low due to the period of collection, the results compared to similar studies in the same region of Ghana indicate that MDA in this area has possibly led to elimination of transmission. The impact of this observation and the analysis of the cibarial armature for the different species are discussed.

395

VECTOR COMPETENCE OF *AEDES ALBOPICTUS* AND *AE. AEGYPTI* TO TWO DIFFERENT CHIKUNGUNYA STRAINS IN THAILAND

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September 2008 saw the beginning of a massive outbreak of chikungunya fever along the Thai-Malaysia border. Over 49,000 cases were reported before this outbreak was finished in late 2009. The recognized vector for chikungunya virus (CHIKV) is the mosquito, *Aedes aegypti*, however *Ae. albopictus* has been recently implicated as the principle vector in several countries. Our goal was to determine the vector competence of both *Aedes* species (AFRIMS lab strain) to two different CHIKV strains; CHIKV historical strain isolated in 1979 and the epidemic strain isolated in 2009. We assessed infection and dissemination rates from orally infected, female mosquitoes at 7 and 14 days post-infection using the SYBR Green I-based real-time RT-PCR. Of the 2 species, CHIKV was only detected in *Ae. albopictus*. In this species, we found over 6 time higher infection and dissemination rates with the 2009 epidemic strain of CHIKV compared to the 1979 historical strain. Our results support previous studies suggesting that *Ae. albopictus* is a capable vector of CHIKV and could easily been responsible for the recent outbreak of chikungunya fever in southern Thailand.

396

TEMPORAL VARIATION IN EXPOSURE TO *ANOPHELES GAMBIAE* AND RISK OF MALARIA TRANSMISSION

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Human biting rates and mosquito infection rates vary across space and time. Mosquito populations also vary temporally, forced by environmental variables such as rainfall, temperature, and humidity. These sources of heterogeneity in the distribution of mosquito populations generate variability in the risk of human infection. Assessment of exposure to malaria vectors is important to our understanding of disease transmission risk, and facilitates planning of control efforts. *Anopheles gambiae* salivary peptide (gSG6-P1) has been designed to enhance its specificity and immunogenicity to detect human exposure to malaria vectors. We evaluated total IgG responses to gSG6-P1 and two malaria antigens (CSP, MSP-119) in an age stratified cohort (< 5, 5-9, >9) from Asutuare, South-western Ghana, an area of relatively low but perennial transmission. 200 randomly selected sera were analyzed from archived samples belonging to a cohort that were followed at 3 contact times (n = 600) as follows; February, toward the end of the dry season, May at the peak of the major rainy season and August a dry period before the minor rainy season representing snap shots of the perennial transmission in the year. Seropositivity above threshold of negative group to the 3 antigens was detected in the cohort at all contact points across age groups. Although, seroprevalence showed temporal trends similar to rainfall and mosquito exposure patterns, specific responses to MSP 1 and CSP, Chi square analysis did not yield significant differences among the different time points. MSP 1 19; 53.3%, 60.6%, 56.7% (chi2= 1.91, p = 0.38), CSP; 21.7%, 19.4% and 23.3% (Chi2 1.53, p =0.46) in Feb, May and August in respective order. In contrast to the above, analysis of seroprevalence and median antibody levels to gSG6-P1 showed significant differences, detecting temporal variations in vector exposure among the cohorts at different time points. Where gSG6 - P1, from Feb, May and August respectively were; 46.2%, 49.7% and 35.7%, (chi2= 7.41, p= 0.02). Repeated measures ANOVA as well as post hoc Tukey multiple comparison test showed significant difference in antibody levels in mosquito exposure between the peak rainfall and dry period preceding minor rainy season. It is concluded that gSG6-P1 is robust and sensitive to detect temporal changes in human exposure.

397

THE RELATIONSHIP OF LAND COVERS WITH ANTHROPOPHILIC *ANOPHELES* SPECIES DIVERSITY AND COMPOSITION IN TWO MALARIA ENDEMIC REGIONS OF COLOMBIA

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The influence of land cover on the abundance, composition and diversity of anthropophilic anophelines was evaluated in six localities of two Colombian malaria endemic regions, the Urabá-Bajo Cauca-Alto Sinu (UCS) and Pacific (PAC) regions, from November 2008 to June 2010. Also, for each site, supervised classification of the types of land cover was performed using the land cover diversity index (SHDI), satellite imagery Landsat7-TM and ground-truthed data. A total of 9,839 specimens were collected that corresponded to 10 species. *Anopheles darlingi* and *Anopheles* nuneztovari were the most abundant species (47.21% and 40.47%, respectively). There was a significant negative relationship between anopheline species diversity and land cover types. The analyses showed localities with high anopheline diversity and low SHDI and others with reduced anopheline diversity and high SHDI. In particular, land cover diversity, type of coverage and pluviosity strongly influenced the distribution of anopheline communities. The presence of An. nuneztovari was correlated with grass cover and bare soils, while An. *darlingi* was correlated with forested cover. These results indicated that the diversity in land cover and some climatic variables contributed to the observed variation in anopheline community structure. This information can be used for the design of more specific vector control strategies in malaria endemic regions of the country.

398

MALARIA VECTOR BIOMICS IN MUTASA DISTRICT, ZIMBABWE

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In December 2012, an International Centers of Excellence in Malaria Research (ICEMR) project began in Mutasa District, Zimbabwe. Recently, Mutasa has experienced a surge in malaria cases despite the use of insecticide treated bed nets (ITNs) and other control efforts. Preliminary research using morphological identification of mosquitoes obtained through human landing catches and CDC light traps suggests that the primary malaria vector in the region is *Anopheles funestus* senus lato. Our aims are to elucidate the true nature of the vector biomics in regard to malaria transmission in Mutasa District, Zimbabwe. Molecular diagnostics to accurately identify vector species, blood meal analysis to determine feeding behavior, and insecticide resistance assays will be utilized to

examine the role of these mosquitoes in malaria transmission. The role of mosquitoes in the transmission of malaria is an important component in eventual control and elimination of the disease.

399

LONG TERM IMPACTS OF COMBINED SEWER OVERFLOW REMEDIATION ON WATER QUALITY, MOSQUITO POPULATION DYNAMICS AND WEST NILE VIRUS AMPLIFICATION

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Combined sewer systems are a significant source of urban water pollution due to the overflow of minimally treated sewage into natural streams (aka a combined sewer overflow, CSO). CSOs contribute to the impairment of natural waterways and are also associated with increased mosquito productivity and elevated risk of West Nile virus (WNV) transmission. We longitudinally investigated the impact of CSOs on water quality, immature mosquito productivity and WNV infection in the city of Atlanta, Georgia, one year before and four years after CSO-facility remediation. Water quality (ammonia, phosphate, nitrate and dissolved oxygen concentrations), immature and adult mosquitoes, WNV infection in mosquitoes, water temperature and rainfall were quantified biweekly between June-October at two urban creeks during 2008-2012. Generalized estimating equations quantified the factors explaining the elevated mosquito productivity at CSO streams and the long term impacts of CSO facility remediation on mosquito productivity and WNV amplification. Nutrient concentrations and late immature (IV-instar and pupae) mosquito populations were significantly higher in CSO than in non-CSO creeks. CSO facility remediation significantly improved all water quality estimates and reduced the number of overflows, mosquito productivity and the overall contribution of CSO-affected streams as sources of WNV infected mosquitoes. The quality of water in CSOs provided a suitable habitat for immature mosquitoes. Remediation of the CSO facility through the construction of a deep storage tunnel improved water quality indices and reduced the productivity of mosquito species that can serve as vectors of WNV.

400

COMPARISON OF THE IFAKARA TENT TRAP AND THE HUMAN LANDING CATCH FOR MOSQUITO COLLECTION IN A SUDAN SAVANNAH AREA OF MALI

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Assessment of *Wuchereria bancrofti* transmission rates by vector sampling is a crucial component of mass drug administration programs to eliminate lymphatic filariasis in Africa. The most common mosquito collection method in current use, Human Landing Catch (HLC), is associated with ethical issues. We have previously reported on the suitability of the ITTC (Ifakara Tent Trap type C) as an alternative method for sampling filariasis vectors in Mali, particularly in settings where *Anopheles* vector density is high.To further assess the utility of ITTC, additional testing was performed in areas of high and low vector density in 2 villages of the Sikasso District in Mali. Mosquitoes were collected every month at three different sites per village from July to December, 2012. The sites in each village were ≥100 meters apart, and the three methods were implemented concomitantly at each site with one ITTC trap and one HLC unit that consisted of one room with two collectors- one inside and the other outside the room. The total number of *Anopheles* collected by HLC was 4,149 in 2012; this was similar to the number collected using the ITTC (3,580 *Anopheles* collected; p=0.094, Mann-Whitney). The numbers of mosquitoes captured using each of the two collection methods in 2012 were highly correlated in Bougoula (r=0.83; p=0.042; Spearman rank), Boundioba (r=0.94; p=0.0048) and when the villages were considered together (r =0.83, p=0.042). In contrast, in 2011 in Boundioba, at a time when the vector density was low (only 251 anopheles were collected by HLC as compared to 682 in 2012), the correlation between the HLC and the ITTC was not significant (r=0.61; p=0.19). From a simple linear regression using the monthly collection data from both years, the HLC yield for *Anopheles* was estimated at [11.04±37.28] + [(1.15± 0.11) x ITTC yield]. This model had a slope that significantly deviated from zero ($p<10^{-3}$) and $r^2=0.84$. In conclusion, when vector density is high, yields from ITTC collection can be used to estimate yields from HLC, allowing comparison of new datacollected without risk to collectors, to historical data.

401

SOMATIC *WOLBACHIA* DENSITIES ARE LOW AND VARIABLE AND UNLIKELY TO INCREASE HOST RESISTANCE TO WEST NILE VIRUS INFECTION IN FIELD POPULATIONS OF *CULEX QUINQUEFASCIATUS* AND *CX. PIPIENS*

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The endosymbiotic bacteria *Wolbachia pipientis* infects a wide variety of insect species and can cause both reproductive phenotypes and increased viral resistance in its host. *Wolbachia* naturally infects *Culex quinquefasciatus* and *Cx. pipiens*, mosquitoes that are important vectors of West Nile virus (WNV). The native *Wolbachia* infections in these mosquitoes could potentially increase the mosquito's resistance to viral infection and thereby reduce vector competence. We recently demonstrated that native *Wolbachia* infections increase host resistance to WNV infection in laboratory colonies of the fruit fly *Drosophila melanogaster* and in *Cx. quinquefasciatus*, reducing the ability of *Cx. quinquefasciatus* to transmit WNV. Using quantitative PCR, *Wolbachia* densities were measured in the ovaries and somatic tissues of individual flies and mosquitoes from laboratory colonies and from field populations of *Cx. quinquefasciatus* and *Cx. pipiens*. *Wolbachia* densities were significantly higher in *D. melanogaster* than in *Cx. quinquefasciatus*, consistent with *Wolbachia* density determining the relative strengths of WNV resistance in these two species. *Wolbachia* densities in somatic tissue in field populations of *Cx. quinquefasciatus* and *Cx. pipiens* were significantly lower than the densities in the laboratory colony of *Cx. quinquefasciatus* originally used to demonstrate *Wolbachia*-induced resistance to WNV, and somatic *Wolbachia* densities equivalent to those measured in field populations did not inhibit infection by WNV. These results suggest that native *Wolbachia* infections in field populations of *Cx. quinquefasciatus* and *Cx. pipiens* are too low to increase host resistance to viral infection and are unlikely to reduce the competence of these mosquitoes to transmit WNV.

402

GENETIC CONTROL OF *AEDES AEGYPTI*

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Mosquito-borne diseases, such as dengue fever, chikungunya and malaria, are major and increasing international public health concerns. The two main vectors of dengue are *Aedes aegypti* and *Ae. albopictus*, both notoriously difficult to control with current methods. We are developing genetic control tools to augment current methods, especially RIDL, an advanced derivative of the classical Sterile Insect Technique (SIT). In a RIDL

control programme 'sterile' male mosquitoes (male mosquitoes do not bite or transmit disease) are released continually over a wide area to mate with the target pest population; death of progeny due to inheritance of the RIDL transgene leads to decline of the target population. A key advantage is that the method is 'self-limiting'; making it controllable, reliable and reversible, in contrast to methods where the genetic change needs to persist in the wild population. Trials in the Cayman Islands in 2009 and 2010 proved the technology could reduce an *Ae. aegypti* population (80% reduction in ovitrap index despite ongoing immigration from adjacent areas). Further trials in 2011 and 2012 in Brazil provided equally positive results in very different ecological and social settings - 80% reduction in ovitrap index and even higher reduction in adult presence in a non-isolated high-density urban area and effective elimination in a more isolated village. Simulation modelling, as reported previously, indicates that even 80% suppression would be sufficient to prevent epidemic dengue in many transmission settings. This Brazilian programme is now expanding. With the smaller scale experiment conducted in Malaysia in 2010, releases in these three countries have confirmed the suitability of the modified males in terms of survival, dispersal, mating competitiveness against their wild counterparts and overall improved fitness compared to irradiated mosquitoes. Knowledge gaps include how best to integrate this genetic method with current approaches in an optimized integrated vector management system.

403

OVERT DIABETES MELLITUS AMONG NEWLY DIAGNOSED UGANDAN TUBERCULOSIS PATIENTS: A CROSS SECTIONAL STUDY

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There is a documented increase of diabetes mellitus in Sub Saharan Africa, a region where tuberculosis is highly endemic. Currently, diabetes mellitus is one of the recognised risk factors of tuberculosis. No study has reported the magnitude of diabetes mellitus among tuberculosis patients in Uganda, one of the countries with a high burden of tuberculosis. This was a cross-sectional study conducted among 260 consenting adult patients with a confirmed diagnosis of tuberculosis admitted on the pulmonology wards of Mulago national referral and teaching hospital in Kampala, Uganda to determine the prevalence of diabetes mellitus and associated clinical factors. Laboratory findings as well as the socio-demographic and clinical data collected using a validated questionnaire was obtained. Point of care random blood sugar (RBS) testing was performed on all the patients prior to initiation of anti tuberculosis treatment. Diabetes mellitus was diagnosed if the RBS level was ≥ 200mg/dl in the presence of the classical symptoms of diabetes mellitus. The prevalence of diabetes mellitus among the admitted patients with tuberculosis was 8.5%. Only 5 (1.9%) patients with TB had a known diagnosis of diabetes mellitus at enrollment. Majority of the study participants with TB-DM co-infection had type 2 diabetes mellitus (n=20, 90.9%). At bivariate analysis, raised mean ALT concentrations of ≥80 U/L were associated with DM (OR-6.1, 95% CI 1.4-26.36, p=0.032) and paradoxically, HIV co-infection was protective of DM (OR-0.32, 95% CI 0.13-0.79, P=0.016). The relationship between DM and HIV as well as that with ALT remained statistically significant at multivariate analysis (HIV: OR- 0.17 95%CI 0.06-0.51, p=0.002 and ALT: OR-11.42 95%CI 2.15-60.59, p=0.004). This study demonstrates that diabetes mellitus is common among hospitalized tuberculosis patients in Uganda. The significant clinical predictors associated with diabetes mellitus among tuberculosis patients were HIV co-infection and raised mean serum alanine transaminase concentrations.

404

PREVALENCE OF *LEGIONELLA PNEUMOPHILA* ANTIBODIES IN DENTAL PRACTITIONERS, 2002-2009

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This is an analysis of the association between the presence of *Legionella pneumophila* antibodies and characteristics of dental hygienists, assistants, and dentists. *L. pneumophila* antibodies were determined via serum assay. All other information was collected via self-administered paper survey. Bivariate analysis was performed to investigate the association between *L. pneumophila* antibodies and characteristics. Multivariate logistic analysis was used to control for possible confounders and effect modifiers. Geographical location, time spent practicing dentistry per year, age, and race were statistically significant predictors of variation of *Legionella* antibody seroprevalence. Antimicrobial methods utilized on dental unit water did not predict *Legionella* antibody prevalence, nor did the frequency with which dental unit water quality was monitored. This suggests that the water quality monitoring and infection control procedures currently in use by dental practitioners may not be sufficient to prevent *L. pneumophila* growth in dental unit water lines.

405

TUBERCULOSIS DRUG RESISTANCE AND THE UTILITY OF GENEXPERT SYSTEM IN AN URBAN SETTING IN INDIA

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India has the highest burden of Tuberculosis cases globally and a third of the population has latent TB infection. Diagnosis of tuberculosis using conventional methods such as sputum microscopy has always been a challenge, and low rates of detection contribute to the emergence and increase in drug resistance. Recent reports have suggested that the resistance to rifampicin, which is highly correlated with multi-drug resistance, ranges from 20-74% in urban centers such as Delhi and Mumbai in India. The availability of the GeneXpert system has made it easier to diagnose TB and detect drug resistance to rifampicin at the same time. In this study, we compared the results of the GeneXpert TB system with sputum microscopy and assessed the prevalence of resistance to Rifampicin among 170 consecutive patients presenting with symptoms suggestive of TB in Bangalore, India. The mean age of the patients was 39 (±11) years and 77% were male. 16% of the patients reported having received treatment for TB previously, 5% were HIV-infected, and 8% also had diabetes. Compared with the GeneXpert system (n=112), sputum microscopy detected 20% fewer patients (95% CI: 13%, 28%) with TB. We also found that only 5% of the patients had a resistance to rifampicin using the GeneXpert system. Our results suggest that the GeneXpert system outperforms sputum microscopy even in a large urban center in India. The results also indicate that the epidemic of tuberculosis and drug resistance varies in different parts of the country, highlighting the need for active epidemiological surveillance and detection of hot spots using geographic information systems to direct and maximize utilization of limited resources.

406

CHARACTERIZING CROSS-RESISTANCE TO FLUOROQUINOLONES IN *MYCOBACTERIUM TUBERCULOSIS*

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The global control of tuberculosis (TB) has been complicated by the global HIV epidemic, the increasing prevalence of multidrug-resistant (MDR) TB and the emergence of extensively drug resistant (XDR) TB. The fluoroquinolones (FQ), levofloxacin (L), gatifloxacin (G) and moxifloxacin (M), are the most effective drugs for the treatment of MDR TB. However, cross resistance between ofloxacin (O) and the more potent, later generation FQs, L, G and M, has not been defined. Definition of cross-resistance patterns will play a crucial role in maximizing the potential of FQs in the treatment of MDR and XDR TB patients, monitoring resistance trends, preventing the emergence of resistance, contributing to the discovery of new anti-tuberculous agents and improving the structure of novel FQs. This study aims to determine the relationship between cross-resistance among FQs and specific mutations in *gyrA/B*. The Quinolone resistance determining regions of *gyrA* and *gyrB* of 109 consecutive FQ resistant clinical *Mycobacterium tuberculosis* isolates collected from Pham Ngoc Thach Hospital TB reference laboratory for Vietnam have been sequenced. Mutations in these regions account for over 80% of resistant clinical isolates from Vietnam. 49 representative isolates of this collection including nine novel gyrB mutants were characterised for their minimum inhibitory concentration (MIC) for O, L, G and M on automated liquid MGIT 960™ system. Different mutations showed variation in the MIC to the 4 tested FQs. Non-*gyrA/B* mutants showed borderline MICs to all 4 drugs. Single A90V-*gyrA* mutants show intermediate MICs to O, L but very low MICs to G, M whereas combinations with other mutations in *gyrA/B* confer high MICs to all FQs. Mutations at codon 94 of *gyrA* confer high MICs while variation in MIC level was observed dependent on the amino acid change at this site. Single novel mutations in *gyrB* show variations in their MIC level. Resistance level for FQs varies by mutation in *gyrA/B*. This project received support from the Wellcome Trust and the International Society for Infectious Diseases.

407

EVALUATION OF A MOLECULAR DIAGNOSTIC PLATFORM FOR SIMULTANEOUS DETECTION OF MULTIPLE RESPIRATORY PATHOGENS IN THAILAND

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Acute lower respiratory tract infections (ALRI) are a leading cause of morbidity and mortality globally, resulting in 2 million deaths in children aged <5 years annually. Testing for multiple viruses and bacteria has historically required multiple individual assays, which can be resource intensive and delay reporting of test results. The TaqMan® Array Card (TAC) is a molecular diagnostic platform that allows simultaneous detection of 30 viral and bacterial respiratory pathogens. We tested 90 stored nasopharyngeal swab specimens from patients hospitalized with ALRI in rural Thailand in 2011. Specimens were originally tested at the Thailand National Institute of Health by real-time RT-PCR (rRT-PCR) single-plex CDC assays for influenza A and B, respiratory syncytial virus (RSV), adenovirus, and human metapneumovirus (HMPV). Specimens for TAC testing were selected from patients who originally tested positive for at least 1 of these 5 viruses (n=85) plus 5 patients that had tested negative; 34 (38%) had been positive for >1 virus. After total nucleic acid extraction, specimens were tested by TAC in parallel with rRT- PCR for these 5 viruses. Using real-time PCR as the gold standard, the sensitivities of TAC were 100% (27/27) for influenza A, 100% (18/18) for influenza B, 100% (15/15) for adenovirus, 90% (9/10) for RSV, and 87% (20/23) for HMPV. The specificity of TAC was 100%. In addition to these 5 viruses, TAC testing detected other viruses or bacteria in 54 (60%) of 90 specimens. Overall, 68 of 90 were positive for >1 pathogen, including 49 with both bacteria and viruses detected. Our findings demonstrate the high sensitivity and specificity of TAC in a field setting for 5 common respiratory viruses compared with individual rRT-PCR assays. TAC could be a useful platform for rapid and simultaneous detection of multiple pathogens in certain settings such as outbreak investigations.

408

ADVERSE REACTIONS OF ANTI-TUBERCULOSIS DRUGS: A RETROSPECTIVE STUDY IN PHAM NGOC THACH HOSPITAL

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Adverse drug reactions (ADRs) due to anti-tuberculosis therapy can affect on tuberculosis (TB) treatment adherence and outcome. In Vietnam, data of anti-TB-induced ADRs are limited. This study was undertaken to describe ADRs to antituberculous agents among in-patient admissions at a large TB reference hospital. A retrospective study reviewed 315 records of all TB patients with ADRs admitted to Pham Ngoc Thach Hospital, Ho Chi Minh City, Vietnam in 2010. Of the 315 patients admitted with suspected antituberculous therapy-associated ADRs, there were 203 (64.4%) males and 112 (35.6%) females; mean age was 46 years old; 80 (25.4%) patients had a previous history of TB treatment; 129 patients (41%) had co-morbidities including HIV-infection (46 cases, 35.7%), diabetes (20 cases,15.5%). Median time interval from initiation of TB treatment to experiencing ADRs was 27 days. The majority of detected ADRs were attributed by physicians to rifampicin (88/315, 27.9%), pyrazinamide (81/315, 25.7%), streptomycin (78/315, 24.8%). Isoniazid and ethambutol were suspected less frequently (43/315, 13,7% and 33/315, 10,5%, respectively). 37 patients (11.8%) had ADRs associated with the second line TB drugs, 90 patients (28.6%) the drugs causing ADR were not established. There were 131 patients (41.6%) had ADRs with a single TB drug, 29.8% patients had ADRs with between 2 to 6 TB drugs. The highest percentage of ADRs (149/315, 47.3%) causing hospital admission was skin reactions; following hepatitis ADRs (29.3%); then nausea, vomiting, abdominal pain (51/315, 16.2%). Anti-tuberculosis drugs were rechallenged in 177/315 (56.2%) patients with different doses, of whom 128/177 (72.3%) developed re-introduction reactions, 26/177 (14.7%) had no recurrence of ADR symptoms and the remaining (13%) had unclear reactions. 34%patients were discharged without prescription for TB therapy.In conclusion, these findings describe anti-TB-induced ADRs in a TB hospital. It is necessary to develop a prospective evidence base and a clinical aligorithm to guide clinicians in the introduction of anti-TB drugs after the development of ADR in patients with TB. This study demonstrates that current approches are highly variable and not based on good evidence.

409

INCIDENCE OF RESPIRATORY TRACT INFECTIONS IS INCREASING EVEN IN AREA LOCATED 2200 METERS ABOVE SEA LEVEL IN KENYA

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Respiratory tract infections (RTIs) are the most common cause of morbidity in African countries and may be accompanied with severe complications. About 2 million of children die every year in the world and more than 40% of these deaths are attributable to Africa. In this study, we have screened clinical records of all patients treated at Ladislaus Batthanyi Strattmann Clinic and diagnosed with RTI. Diagnosis of RTI was based on clinical examination. We have noticed, that RTI were the most prevalent infection among all patients treated in abovementioned clinic, counting for 40,48% in average. However, interesting increase of prevalence of RTI was observed, with 21,41% proportion in 2009 and 30,22% in 2010, then arising above 50% in 2011 and 2012 (52,11% and 58,17% respectively). RTI are still dominating cause of morbidity among patients treated in Eldoret in Kenyan highlands, and interestingly they are becoming even more prevalent than in past. This may be assumed to better diagnostic tools, what allow physicians to differentiate RTI that would otherwise be misdiagnosed as malaria.

410

RESPIRATORY VIRUSES IN CHILDREN WITH AND WITHOUT RESPIRATORY SYMPTOMS IN PERU

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Viral respiratory infections are a primary cause of illness in developing countries, disproportionately affecting young children. Some of the most widely described viral respiratory pathogens in children are respiratory syncytial virus, influenza virus, adenovirus, metapneumovirus, rhinovirus and coronavirus. Despite vast information about these respiratory viruses' characteristics in symptomatic disease, sparse data exists about their presence in those without symptoms. The aim of this study was to use real time PCR to indentify respiratory viruses in symptomatic and asymptomatic children in three large referral hospitals in Lima, Peru. We also investigated the relationship between the presence of disease and a specific virus strain. Cases were defined as individuals five years old or younger with five days or less of influenza-like illness symptoms, defined as fever (≥38°C) and one or more of the following: sore throat, cough, or runny nose. Controls were age and gender matched individuals attending the same health facility without respiratory complaints in the previous two weeks. Nasopharyngeal samples were analyzed using real time PCR and low-density array cards. Positive samples underwent sequencing for strain identification and phylogenetic analysis. Our preliminary results show that some respiratory viruses, such as respiratory syncytial virus, have a high frecuencie of detection in asymptomatic subjects, suggesting that identification of a virus does not always indicate causation of disease.

411

TB PERICARDITIS: IS THERE A ROLE FOR INTERFERON-Γ RELEASE ASSAYS IN IMPROVING DIAGNOSTICS? A CASE REPORT AND REVIEW OF THE LITERATURE

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A 14 year old male Vietnamese immigrant presented with 2 weeks of fever, shortness of breath, cough, chest pain, and weight loss; having last visited South East Asia 2 years ago. He had tachycardia, jugular venous distention, pericardial friction rub, and hepatosplenomegaly. Chest x-ray showed an enlarged heart; echocardiogram revealed pericardial effusion with cardiac tamponade; and he underwent pericardiocentesis. Gram stain, aerobic/anaerobic culture, fungal stain/culture, and acid fast bacilli (AFB) stains were negative from pericardial fluid. HIV antibody screen was negative. He was discharged with a diagnosis of viral pericarditis, but was readmitted 3 weeks later due to persistence of symptoms. Tuberculosis (TB) skin test (TST) was negative, but an interferon-γ release assay (IGRA) (QuantiFERON-TB Gold) was positive. He improved following empiric TB therapy and adjunctive steroids. The diagnosis of TB pericarditis was subsequently confirmed with positive pericardial fluid culture for *Mycobacterium tuberculosis* complex, pan-susceptible. TB pericarditis is a rare manifestation of TB, uncommonly seen in the U.S. The diagnosis can be challenging, as TB may not be initially considered, and available TB diagnostics have modest sensitivity. Early diagnosis requires a high index of suspicion; especially for those with an increased background risk for TB infection. In our case, while the TST was negative, the simultaneously drawn IGRA was positive. The role of IGRAs in the diagnosis of TB pericarditis is speculative, as there is a lack of data on the sensitivity and specificity in this particular setting. It should be emphasized that TSTs and IGRAs do not lead to the actual diagnosis of TB disease, but rather heighten our index of suspicion for TB infection. The discordance between TST and IGRA in establishing latent TB infection has been increasingly recognized, and the TST-negative IGRA-positive result may simply be indicative of higher sensitivity of the IGRA. The exact immunological mechanism, however, has not been established.

412

DISTRIBUTION OF HEALTHCARE RESOURCES FOR INFLUENZA PANDEMIC RESPONSE IN CAMBODIA

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Human influenza infection poses a serious public health threat in Cambodia, a country at high risk for the emergence of future pandemic influenzas. Prior pandemics, including H1N1, demonstrated the considerable adverse impact of influenza on poor communities in developing countries as well as the limitations of health system capacity for pandemic response. Investigation of healthcare resource distribution and inequalities can inform decisions regarding health resource mobilization and investment for pandemic influenza mitigation. A health facility survey was performed across Cambodia to obtain data on availability of five key resources (inpatient beds, doctors, nurses, oseltamivir, and ventilators) important for pandemic influenza response. Distributions were analyzed at the Operational District (OD) and Province levels, expanding on prior research that focused solely at the latter. A summary index of resource distribution inequality was calculated (Gini coefficient) and a potential link between socioeconomic status and resource distribution, not addressed in earlier studies, was explored by mapping resource densities against poverty rates. Gini coefficient calculation revealed variable equality in distribution of the five key resources at the Province and OD levels. A

greater percentage of the population resides in areas of relative under-supply (28.5%) than relative over-supply (21.3%). Hospital-based inpatient beds, doctors, and nurses are most heavily concentrated in wealthier areas; however, concentrations of non hospital-based inpatient beds and nurses increase alongside poverty level. The considerable heterogeneity in healthcare resource distribution across Cambodia suggests that mobilization of selected resources or patients across ODs could be beneficial in the event of a pandemic influenza. More broadly, these findings may inform future health resource investment in Cambodia, both for pandemic preparedness and general health system strengthening.

413

EPIDEMIOLOGY OF CHILDHOOD PNEUMONIA AND NASOPHARYNGEAL VIRAL CARRIAGE IN RURAL NORTH PAKISTAN, APRIL 2012 - MARCH 2013

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Previous work demonstrated that pneumonia was the main cause of childhood mortality in Oshikhandass village, Gilgit, from 1989-1996. A follow-up study is on-going to determine changes in pneumonia epidemiology, response to antibiotics and carriage of viral pathogens. Female health workers trained in pneumonia management using WHO IMCI guidelines conducted weekly surveillance of children <5 years. Each episode was classified by severity and treatment or referral given. From December 2012-March 2013, nasopharyngeal (NP) swab samples obtained from children with pneumonia were tested for the presence of 20 respiratory viruses by PCR-based analysis. An average of 804 children were followed from April 2012-March 2013 with 213 episodes detected (incidence 27 /100 child years, 95% CI, 26.6-27.4, average age 22 months). Of these, 182 episodes occurred from December 2012-March 2013, (winter incidence 69/100 child years, 95% CI, 68.6-69.4); 62% of all episodes occurred in January-February. Most cases (96%) were categorized as non-severe; most children (69%) were treated with amoxicillin. Treatment failure after 3 days occurred in 10% of cases, with 1% experiencing a worsening of symptoms and 9% reporting no change; 6 needed hospitalization and none died. PCR results were available for 130 pneumonia episodes (71%). The frequency of viruses found was: enterovirus/rhinovirus (45%), respiratory syncytial virus (18%), coronavirus (229E, OC43, C44) (11%), metapneumovirus (11%), adenovirus (5%), influenza A (3%) parainfluenza 1 and 4 (3%) and bocavirus (1%). At least one viral pathogen was present in 73% of samples and mixed infections were found in 22%. We conclude that most pneumonia occurred during 2 winter months and that NP carriage of respiratory viruses is high. Public health measures to prevent spread of respiratory viruses need to be intensified just before winter season. Viral pathogens may be substantial contributors to community acquired WHO-defined pneumonia and further studies on appropriateness of antimicrobial treatment are warranted.

414

FASCIOLA HEPATICA INFECTION IN AN INDIGENOUS COMMUNITY OF THE PERUVIAN JUNGLE

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In South America, fascioliasis is considered an illness mostly restricted to the highlands. Human fascioliasis has not been reported in the Peruvian jungle. We report 5 probable cases of autochthonous *Fasciola hepatica* infection in an indigenous population from a remote area of Manu National Park. A community intervention for soil transmitted helminths was carried out in Yomibato. Stool samples from 215 subjects were obtained and preserved in 10% formalin and 96% alcohol. These were transported to the laboratory at the Universidad Peruana Cayetano Heredia - University of Texas Medical Branch Collaborative Research Center in Cusco for parasite testing. Direct, rapid sedimentation, and Kato Katz tests were performed in all samples. Five subjects (2.3%) were identified as having eggs with the morphological appearance of *Fasciola hepatica* in the rapid sedimentation and Kato Katz tests. The mean number of eggs/gram of stools was 40 (±28.3). The mean age of the subjects was 15.5 (±6.3) years, 4 were female, 1 had significant anemia, and 2 had stunting. Four subject were co-infected with other helminths or protozoa: 2 had *Trichiuris*, 1 hookworm, 1 *Giardia*, and 2 *Blastocystis hominis*. None had a history of living in or consuming vegetables coming from the highlands. All subjects had received 4 doses of albendazole in the previous 3 months. RT- PCR with primers targeting the trematode *18s rRNA* gene and the *Fasciola hepatica ITS-1* region was performed on the stool samples. DNA from adult *Fasciola* parasites and stools from a *Fasciola* infected patient in Cusco (altitude 3,400 m) were the positive controls. In 3 subjects, the trematode *18s rRNA* PCR showed amplicons with melting curves similar to those from the positive controls. Two of these subjects also had a positive RT-PCR test for the *Fasciola hepatica ITS-1* region. This is the first report of *Fasciola hepatica* infection in 2 indigenous subjects from a remote area of the Peruvian jungle. Further epidemiological studies may be required to confirm the presence of *Fasciola* and its secondary hosts in the region.

415

FASCIOLIASIS IN PAUCARTAMBO-CUSCO: SIGNIFICANT PREVALENCE VARIATION AMONG COMMUNITIES WITHIN A SMALL GEOGRAPHIC AREA

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Fascioliasis is an important problem in the highlands of Peru. Mass triclabendazole treatment is a proposed control strategy for hyperendemic areas which makes imperative knowing the prevalence of *Fasciola* at the community level. We studied the epidemiology of *Fasciola* in 6 communities in a small geographic area of Huancarani district in Paucartambo, Peru. The prevalence of *Fasciola* and intestinal parasites, anemia, and malnutrition was studied in children 3 to 12 years old receiving mass albendazole treatment. Direct, rapid sedimentation, and Kato Katz tests were performed on at least one stool sample in 290 children (Huacaycancha 23.3%, Huayllapata 19.7%, Piscohuata 16.6%, Ohuay 16.2%, Chinchayhuasi 14.8% and Queunacancha 9%). Mean age was 10 (±2.6) years, 52% were male, mean size of the household was 6.2 (±17) people, and mean school years of the mother was 3.8 (±3). For most (93%) the drinking water source is the community's reservoir, 72% own livestock, and 66% participate in livestock tending activities. Ten percent of children were underweight, 33% were stunted, 2.8% had wasting, and 45% were anemic. *Fasciola* eggs were detected in 9.4%, *Ascaris* in 13.4%, *Hymenolepsis nana* in 10.7%, *Trichiuris* in 1.7%, hookworm 1.4% and *Strongyloides* in 1.4%. *Giardia* was detected in 29% and *B. hominis* in 53%. Overall, 48.6% had at least one parasite, excluding *B. hominis*. Fascioliasis prevalence varied by community: 17.5% Huayllapata, 16.7% Piscohuata, 9% Huacaycancha 4.7% Chinchayhuasi, 3.8% Queunacancha, and 0% Ohuay (p=0.01). *Eosinophilia* (>500 eosinophils/µL) was present in 21% and also varied by community: 48.6% Ohuay, 31.6% Chinchayhuasi, 28% Huayllapata, 10% Huacaycancha, 8.9% Piscohuata, and 0% Queunacancha (p<0.01). Although, the overall prevalence of *Fasciola* in Huancarani district placed it as a meso-endemic area, the community level prevalence range from hypo to hyper-endemic areas within a very small geographic area. The high prevalence of eosinophilia suggests that some children were infected with undetected helminthes (e.g. acute phase of fascioliasis).

416

ANTI-APOPTOTIC EFFECT OF *OPISTHORCHIS VIVERRINI*-THIOREDOXIN-1 (OV-TRX-1) - HUMAN APOPTOSIS SIGNAL REGULATING KINASE-1(ASK-1) IN CHOLANGIOCYTES

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Opisthorchis viverrini is a carcinogenic human liver fluke highly endemic in Thailand, Lao PDR, Cambodia and Vietnam. Infection is acquired by eating uncooked freshwater cyprinoid fish and can induce carcinoma of the bile duct (cholangiocarcinoma). Apoptosis/anti-apoptosis is one of the major mechanisms in the carcinogenesis of this cancer. Our study aimed to investigate apoptotic effects of *O. viverrini* thioredoxin-1 (OV-Trx-1) and Apoptosis signal-regulating kinase-1 (ASK-1) in human cholangiocyte (H69) using oxidative stress model. The recombinant Ov-Trx-1 protein was expressed in *E. coli*, purified on NI-NTA (6xHis tag) column and LPS removal by Triton-X114. H-69 cells were incubated with Ov-Trx-1 for 24, 48 and 72 h and 300 μM hydrogen peroxide was added to induce apoptosis. Harvested cells were measured for apoptosis by Annexin-Valexa488/PI using a Flow Cytometer. Immunolocalization of the Ov-Trx-1 in the cells was done by Immunofluorescence. mRNA expression of apoptosis and anti-apoptosis-related genes (i.e., BAX, ASK1, Caspase9, Caspase8, Caspase3, survivin, Mcl-1, JNK1/2, P38K) was performed by RT-PCR assay. Immunoprecipitation of cell lysates using His-Mag sepharose® bead was analyzed by immunoblotting with specific primary antibodies (anti-ASK-1, anti-His, anti-Ov-Trx-1, anti-Trx-1, anti-beta actin). Ov-Trx-1 showed anti-apoptotic activity in H-69 cells induced by H2O2. Ov-Trx-1 incubated cells showed decreasing mRNA expression of the apoptotic genes compared to controls. Immunolocalization revealed OV-Trx-1 was observed in the H69 cells by immunofluorescence. Immunoprecipitation of cell lysates showed that Ov-Trx-1 could bind to human ASK-1 by immunoblotting. The results suggest that OV-Trx-1 may play an important role in anti-apoptosis mechanism in pathogenesis of liver fluke induced cholangiocarcinoma.

417

WAXING AND WANING PULMONARY CYSTS: A FLUKE?

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Global travel is associated with increasing incidence of helminthic infections in non-endemic regions. We report a case of patient with pulmonary paragonimiasis in Upstate New York who presented with recurrent hemoptysis. 20 year old healthy female college swimmer was referred for evaluation of intermittent episodes of hemoptysis for 1 year. Her symptoms began after a dive 3 meters above the pool resulting in a "belly flop". Complete blood count with differential was within normal limits. Initial chest x ray was normal. CT thorax showed 2 cystic lesions in the right lower lobe. The patient denied alcohol, cigarette or illicit drug use. Travel history was significant for a trip to Costa Rica and Bahamas 1 year prior. She continued to note 1-2 episodes every other week of quarter sized hemoptysis associated with shortness of breath. Bronchoscopy with bronchoalveolar lavage, brushings, and CT guided biopsy results were unremarkable. Cultures from the bronchial washings and the cytology were negative. Pulmonary Function Tests were normal. Workup for connective tissue disorders was negative. Further evaluation for pulmonary TB, mycoplasma, histoplasmosis and blastomycosis was negative. Subsequent CT scans showed interval resolution and redistribution of the cystic lesions. Paragonimus westermani antibody titers were ordered and found to be elevated at 1:32. The diagnosis of chronic pulmonary paragonimiasis was made and the patient responded well to praziquantel. Presentation of hemoptysis after diving with the finding of cysts on chest CT led to a consideration for traumatic pneumatocele. However, given the relatively mild chest injury at the time of the dive, cysts that resolved and recurred and the recent travel history, an infectious etiology was more likely. Although stool, sputum, and bronchial washings for ova and parasites were negative, *Paragonimus westermani* titers were elevated. Stool studies are insensitive and eosinophilia is much less common in chronic paragonimiasis. Serologic testing for anti-*Paragonimus* IgG however has a sensitivity of 100% and a specificity of 91%-100%. Paragonimus westermani also known as the lung fluke, is acquired through the ingestion of raw or undercooked crabs or crayfish and is the most common cause of hemoptysis worldwide. This clinical vignette underscores the importance of health care providers in the United States to recognize common worldwide infections.

418

SOIL-TRANSMITTED DISEASES AND HUMAN FASCIOLIASIS WITH FEVER AND HIGH *EOSINOPHILIA* IN GEORGIA

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Soil-transmitted Helminthiasis and Fascioliasis are the most widespread parasitic diseases in Georgia. Wide geographic distribution makes these diseases as a major health issue in Georgia. Medical examinations to reveal Fascioliasis and helminthiasis cases were conducted in West Georgia during 2007-2011. For this pilot study 10 districts of Imereti region were selected. 1046 residents had high eosinophilia, digestion system complains and fever for several days in anamnesis. The main diagnostic test used for helminthiasis is an ovoscopic examination of stool by Kato-Katz method. Among 1046 individuals 313 (30%) were infected with different types of helminthiasis with 172 (50%) cases representing soil-transmitted diseases. Among those with soil-transmitted diseases 37% had Ascariasis and 12% had Trichuriasis. Human fascioliasis was present in 5% of study participants. The study has shown that historically widespread Ancylostomiasis (Hookworm), caused by Necator americanus and *Ancylostoma duodenale* were not identified among study participants. During epidemiologic investigation WHO experts recorded several species of lymnaedae - intermediate hosts of Fasciola. The study revealed that the prevalence of helminthiasis is high among residents of rural areas (37,5%) and suburbs (20%). The investigation showed that all favorable factors for the spread of soil-transmitted parasitic diseases is present in the Imereti Region and it's necessary to ensure that epidemiologic surveillance, preventive and treatment measures are adequately implemented. In addition, there is an urgent need for better monitoring and control of helminthiasis using new technologies.

419

DEVELOPMENT OF COPRO-ANTIGEN DETECTION OF *OPISTHORCHIS VIVERRINI* USING RECOMBINANT CATHEPSIN F

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Opisthorchis viverrini, a food-borne trematode parasite as a major public health problem in Southeast Asia, particularly in Thailand, Laos, Cambodia and Vietnam. Chronic and repeated infection with *O. viverrini* is associated with the fatal cholangiocarcinoma, a rare cancer of the bile duct. Diagnosis of the infection is conventionally by stool examination. Research indicates that several parasite cysteine proteases have potential as diagnostic candidates. Cathepsin F is a major cysteine protease produced by eukaryotes including human liver flukes. We investigated whether *O. viverrini* Cathepsin F would be suitable for immunodiagnosis of human opisthorchiasis. Recombinant *O. viverrini* Cathepsin F (rOV-CF) was expressed in BL21 (DE3), purified by means of a His Trap column and gradually refolded overnight. rOV-CF was used for immunodiagnosis by Sandwich ELISA. Gaining capture antibody, two 18-week-old egg-laying Gallus domesticus chicken were immunized with 0.5 mg of purified rOv-CF mixed with equal amounts of adjuvant followed by 4 boosts. Eggs were collected and IgY extracted using the PEG 6000 precipitation method. Sandwich ELISA was performed by coating the plate overnight with 100 µl of carbonate-bicarbonate buffer containing 5 µg of purified IgY at 4ºC. The plate was blocked then sequentially incubated with a 2-fold serial dilution of *O. viverrini* somatic antigen and positive and negative samples of hamster feces. Rabbit anti-Ov-IgG from our previous study was used at a dilution of 1:500 (v/v). Peroxidase-conjugated goat anti-rabbit IgG at a dilution of 1:5000 (v/v) was used as the secondary antibody. The color was developed using HRP ELISA substrate and the absorbance was recorded. SDS-PAGE results showed a 40 kDa single band of mature rOv-CF and enzyme activity test verified it as an active cathepsin F. The Sandwich ELISA revealed that chicken IgY was able to capture the cathepsin F as low as 1 µg of *O. viverrini* antigen. Twenty three *O. viverrini* infected hamster feces, but not uninfected ones showed positive results. However, the background absorbance was still high in negative control. Further refinement is in progress. The Sandwich ELISA established in this study yet promising in *Opisthorchis* antigen detection in animal or human faecal samples.

420

TRANSCRIPTOME ANALYSIS OF *PARAGONIMUS KELLICOTTI* MAY LEAD TO DEVELOPMENT OF IMPROVED SEROLOGICAL DIAGNOSTIC TESTS FOR PARAGONIMIASIS

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Paragonimus infections are widely distributed throughout Asia, Africa and the Americas and are among the most prevalent food-borne trematode infections. Following ingestion, parasites penetrate the intestine and make their way to the lung, causing symptoms that range from abdominal discomfort to severe lung disease. Safe and highly effective medications are readily available, but misdiagnosis often results in prolonged suffering and subjection to uncomfortable but ineffective treatment regimes tailored to unrelated illnesses (cancer, tuberculosis, etc). This is particularly common in North America where *P. kellicotti* is a relatively rare infection that physicians are not used to encountering. In most cases, specific IgG antibodies can be detected with the onset of pathological symptoms, often before parasite eggs can be detected in sputum, bronchoalveolar lavage, or stool. Characterization of the parasite antigens that stimulate this immune response would facilitate the design of improved diagnostic tools that could greatly improve patient outcomes. Unfortunately little information about the genome, transcriptome, and proteome of *Paragonimus* is available at this time. In order to address this need, we sequenced the transcriptome of developing adult *P. kellicotti*. Flukes were collected from experimentally infected gerbils 3-6 weeks post infection, and RNA was isolated and sequenced. A total of 120 million 100bp paired-end sequences were generated. High-quality read pairs were assembled into 246,149 consensus sequences. Despite obvious fragmentation, comparison with core eukaryotic genes suggests that approximately 79% of all *P. kellicotti* genes are represented in the initial transcriptome assembly. Translated transcripts will be used to facilitate characterization of peptides from mass spectroscopy analysis in order to identify parasite proteins recognized by the sera of infected patients. We will report on the progress of the improved transcriptome, the genome and the identification of immunodominant proteins that may lead to improved diagnostic tests for Paragonimiasis.

420A

A GRANULIN GROWTH FACTOR SECRETED BY THE CARCINOGENIC LIVER FLUKE, *OPISTHORCHIS VIVERRINI*, AND ITS ROLE IN CARCINOGENESIS

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The human liver fluke, *Opisthorchis viverrini*, infects 9 million people throughout South-East-Asia and is a major cause of cholangiocarcinoma (bile duct liver cancer). In fact, as many as one-sixth of infected patients will develop liver cancer. The mechanisms by which the parasite causes cancer are multi-factorial, but one process is the secretion of mitogenic parasite proteins into the bile ducts, driving cell proliferation and creating a tumourigenic environment. Using proteomics and transcriptomics to characterise the *O. viverrini* secretome, we identified Ov-GRN-1, a homologue of the human growth factor, granulin. Previously we demonstrated potent growth factor activity (nanomolar) of recombinant Ov-GRN-1 on human biliary cells. Here we show that Ov-GRN-1 induces wound closure *in vitro* and results from *in vivo* wound healing experiments are ongoing and will be presented. Ov-GRN-1 stimulated angiogenesis (blood vessel formation) *in vivo*, a process critical for cancer development. Binding of recombinant Ov-GRN-1 to biliary epithelial cells induced dramatic changes in protein expression. Indeed, and in support of our hypothesis, many of the cellular proteins that were upregulated in response to Ov-GRN-1 were associated with cell growth and cancer, whereas the downregulated proteins were associated with tumour/proliferation suppression. Finally, silencing of Ov-grn-1 gene expression using RNA interference resulted in reduced mitogenicity of biliary cells by *O. viverrini* excretory/secretory products. Our novel findings contribute to the understanding of host-parasite interactions, and begin to address the mechanisms by which this parasite causes such a devastating form of cancer.

421

INVESTIGATING THE ROLE OF CATHEPSIN B IN THE FREE-LIVING HELMINTH *SCHMIDTEA MEDITERRANEA* AS A MODEL FOR THE PARASITE *SCHISTOSOMA MANSONI*

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Schistosomiasis, a disease caused by the blood fluke genus *Schistosoma*, affects over 200 million people worldwide, especially in developing

countries. While not often fatal, chronic infection can damage internal organs and delay growth in children. It is known that proteolytic activity in *Schistosoma mansoni* is key to host invasion and worm survival. Characterizing the biological function of these proteases could lead potential drug targets. Unfortunately, direct assays like RNAi are not easily done in *S. mansoni,* making it difficult to determine the role played by specific proteases. However, RNAi can be used in *Schmidtea mediterranea*, a closely related free-living flatworm, which has been widely studied due to its ability to entirely regenerate and remodel its body tissues following injury. Although little is known about the proteome of *S. mediterranea,* its genome has been sequenced and is publicly available. Our aim is to identify *S. mansoni* protease homologs in *S. mediterranea* and determine biological function in both flatworms. After conducting an informatics-based survey of proteases in *S. mediterranea*, we are currently investigating the activity of cathepsin B, a cysteine protease in the papain family. This protease has been suggested as both a potential vaccine target and a serodiagnositc marker in *S. mansoni*, where it is localized to the gut and is involved in digestion of host albumin and hemoglobin. Preliminary data show a possible regeneration phenotype of cathepsin B knockdown in *S. mediterranea*, suggesting that cathepsin activity may be necessary for tissue remodeling. Western blotting has revealed a change in active cathepsin levels after a period of starvation with or without body amputation, another indication of involvement in regeneration and remodeling. It is also likely that cathepsin B performs a role in digestion, although more work is being done to confirm this. We are currently purifying cathepsin B from planaria lysates, as well as generating a specific antibody to determine its localization in *S. mediterranea*.

422

ANTI-*SCHISTOSOMA MANSONI* RESPONSE IN MICROSCOPY NEGATIVE PARTICIPANTS

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The recommended strategy for Schistosomiasis morbidity control in Ghana relies on praziquantel chemotherapy. Though microscopy is a gold standard for diagnosis, it does not detect infection in endemic participants who are no longer shedding eggs because they are trapped in tissues. Schistosomiasis control cannot be separated from effective diagnosis, hence the need to screen sera for epidemiologic transmission markers to define exposure. This study determined anti-*Schistosoma* antibodies (IgM and IgG) to Soluble Egg Antigen (SEA) and Soluble Worm Antigen preparation (SWAP) in microscopy negative participants living in an endemic area. In a larger study, systemic random sampling was done to select 107 negative participants whose stool tested negative for *S. mansoni* ova and their sera screened with Enzyme Linked Immunosorbent Assays (ELISA) to detect anti-IgM and IgG antibodies. Eight microscopy positive sera were included to validate the ELISA test as well as compare SWAP and SEA responses to negative participants.Microsoft excel and SPSS 16 was used to organize data as well as check for statistical differences in microscopy and ELISA test values using chi^2 test at 95% confidence interval. For IgM, IgG and IgM/IgG sera showed 52.3%, 22.4% and 59.8% for SWAP and 31.0%, 46.0% and 53.27% for SEA respectively (N= 107 for each test). Positive sera showed 50%, 88% and 88% for SEA and 62.5, 62.5% and 25% for SWAP IgM, IgG and IgM/IgG respectively, (N= 8 for each test). In effective disease control, serum antibody was useful in defining exposure, grouping acute and chronic infections in negative participants. Responsiveness of sera to SEA and SWAP could be affected by intensity and prevalence.

423

SCHISTOSOMIASIS ELIMINATION: IDENTIFYING STRATEGIES AND PATHWAYS FORWARD

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Current WHO guidelines recommend a dual strategy for the control of schistosomiasis; (1) morbidity control adapted to the country context based upon regular mass drug administration (MDA) of praziquantel (PZQ) in high burden areas, and (2) a non-specific integrated control strategy (including the provision of safe water, adequate sanitation, and snail control) in areas where low endemnicity has been achieved and elimination may be feasible. In January 2012, the London Declaration on Neglected Tropical Diseases called for a coordinated push towards improved control globally, with focal elimination of schistosomiasis by 2020. However, current WHO and national governmental strategies are not aligned with the global goal of targeted elimination and are unlikely to achieve these benchmarks. To achieve focal elimination, the schistosomiasis community must rethink current strategic approaches. We sought to outline current WHO recommended schistosomiasis strategies and compare them to a proposed prevalence-specific elimination strategy. This prevalence-specific strategy is based on the understanding that more intensive MDA programs involving broader coverage and more frequent treatments can cure most infections and prevent reinfection. Moderate or high risk communities will require MDA of PZQ for 4 or more years and communities that attain low-prevalence and no longer require MDA therapy must utilize non-MDA based interventions to achieve complete transmission interruption. Specific snail control, sanitation, and diagnostic recommendations are designed according to the disease prevalence and risk level of a given community. Differences between WHO and the suggested new approaches to schistosomiasis elimination are highlighted and the potential disease reduction impact of both approaches is provided and compared. Specifically, the proposed strategy can be expected to increase successful cure rates, reduce community-level reinfection rates, and prolong longevity of transmission interruption relative to the current WHO strategy.

424

SEROLOGICAL-PROTEOME OF THE PARASITE *SCHISTOSOMA MANSONI*: AN APPROACH FOR DIAGNOSTIC BIOMARKERS IDENTIFICATION

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Despite intensive efforts towards schistosomiasis control, the disease is still one of the most prevalent in the world. Improvements in the diagnostic would represent a step forward to the transmission control, being a suitable diagnostic assay essential for epidemiological surveys. Diagnosis procedures require continuous adaptation to the disease control stage and assays that are simple, inexpensive, sensitive, specific and able to distinguish active from prior infection have yet to be developed. Progress on post-genomic technologies resulted in a more rational approaches for new biomarkers discovery. A total of 47 different immunoreactive proteins were identified by our group using *Schistosoma mansoni* adult worm protein extracts probed with pooled sera of infected (INF), non-infected individuals from endemic area (NE) and of non-infected individuals from non-endemic area (NI), in a two-dimensional Western-blotting assay (2D-WB). Of these proteins, seven reacted exclusively to the INF sera pool, suggesting a possible use of this antigen panel to diagnostic purposes. Western-blotting (WB) with the *S. mansoni* recombinant protein Major Egg Antigen (rSmP40), one of the INF sera pool exclusively recognized antigen, showed a similar serum recognition profile to the native protein in

the 2D-WB. For further validation of the rSmP40 as diagnostic candidate, WB were conducted using the serum samples individually. Of the 12 INF serum samples, 8 (67%) recognized a protein band of approximately 40 KDa, corresponding to the rSmP40. None of the 8 NE (0%) and neither of the 7 NI (0%) serum samples were reactive to this same protein. Once the proteins which make up the panel of exclusively INF sera pool recognized antigens were identified simultaneously in a same 2D-WB, it is proposed that all of them might have the same potential as the rSmP40 for the development of a new diagnostic test. These antigens may be used as a diagnostic kit based on the detection of at least one of them, being capable to distinguish the clinical status of the schistosomiasis endemic area residents.

425

IMMUNE RESPONSES TO MEASLES, MUMPS AND RUBELLA (MMR) VACCINATION AMONG SCHISTOSOMIASIS AND GEOHELMINTH INFECTED SCHOOLCHILDREN IN LEYTE, PHILIPPINES

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Schistosomiasis is a chronic and debilitating disease caused by helminths of the genus *Schistosoma*, affecting millions in the developing world including the Philippines. Helminths, including schistosomes, may suppress immune responses to evade attack, and we describe a study investigating immunoregulation of the measles, mumps and rubella (MMR) vaccine by schistosomiasis and helminth infections among schoolchildren in Leyte, Philippines. N=104 schoolchildren aged 7-16 from a highly endemic village for schistosomiasis consented and provided stool samples and blood pre- and post- MMR immunization. Kato Katz examination revealed that 38% of the cohort was infected with schistosomiasis, while 73%, 86%, and 20% were infected with *Ascaris*, *Trichuris*, and hookworm, respectively. Whole blood culture supernates of pre- and post-MMR immunization blood and stimulated with MMR antigens were assayed for 11 cytokines: Interleukin (IL)-1, IL-2, IL-6, IL-8, TNFα, IL-12, Interferon γ, IL-4, IL-5, IL-13 and IL-10. In multivariate regression analysis, schistosomiasis infection was associated with increased IL-1 (p=0.02) and IL-8 (p=0.05) levels in MMR-stimulated supernates collected at baseline. However, neither schistosomiasis nor geohelminth infections were associated with cytokine responses post-MMR immunization. Robust Th1 and pro-inflammatory cytokine response were observed at this timepoint, consistent with vaccination for viral antigens. Preliminary results of MMR-specific antibody responses by ELISA suggest that schistosomiasis or geohelminth infection were not associated with differences in post-vaccination antibody responses. Overall, these data suggest that schistosomiasis potentially enhances pro-inflammatory responses to bystander antigens (MMR), however MMR vaccine-induced antibody and cytokine responses may not be altered by schistosomiasis or geohelminth infection. Further data on neutralizing antibody responses by the measles plaque reduction neutralization test will be presented.

426

BIOCHEMICAL CHARACTERIZATION OF SERINE PROTEASE INHIBITORS FROM *SCHISTOSOMA JAPONICUM* AS NOVEL TARGETS FOR PUBLIC HEALTH INTERVENTIONS

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Schistosomiasis remains a major global public health problem, but despite significant progress in the control of this disease, clear limitations necessitate the development of an effective anti-schistosomal vaccine. Serine protease inhibitors (serpins) are a superfamily of proteins involved in many important biological processes such as blood coagulation, fibrinolysis and inflammation. These inhibitors are known to play central roles in host immune modulation and/or evasion by pathogens. Furthermore it has been suggested that serpins may have evolved specifically in limiting the host immune activation by mediating the inhibition of host immunomodulatory signals. We hypothesise that serpins provide similar functions for schistosomes and their disruption by an effective host immune response by vaccination provides an opportunity to eliminate and control these parasites. Therefore, the aim of this study was to biochemically characterise novel serpins from *Schistosoma japonicum* and investigate the potential of these proteins as suitable anti-schistosomal vaccine candidates. Gene expression results from data mining of previously published microarray findings of our group and subsequent confirmation with quantitative PCR showed that two *S. japonicum* serpins termed *SjB6* and *SjB10* are differentially expressed in different life cycle stages of the parasite. The highest relative gene expression was observed in the egg and cercarial stages for *SjB6* and *SjB10* respectively indicating possible pathological and/or immunological relevance as well as a possible role for SjB10 in cercarial penetration. Western blot analysis confirmed the expression of the native proteins in the adult worms. Recombinant proteins produced were tested for their inhibitory activity against a panel of serine proteases. SjB10 was shown to be biochemically active against pancreatic elastase and chymotrypsin while SjB6 showed no activity against any of the proteases tested. Work is now ongoing for the immunolocalisation of the native proteins using polyclonal antibodies raised in rabbit as well as evaluating their possible anti-schistosomal vaccine efficacies.

427

T LYMPHOCYTE PROFILE AND ACTIVATION STATUS IN SCHISTOSOMIASIS PATIENTS WITH LIVER FIBROSIS

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Schistosomiasis takes the second place in clinical and epidemiological magnitude among parasitic diseases in the world. The host immune response is crucial for the protection and pathology. Our hypothesis is that an impaired T regulatory response plays a role in the development of periportal fibrosis in human schistosomiasis. In this study we evaluate the phenotype and the degree of T cell activation in schistosomiasis patients with periportal fibrosis. We included 37 subjects living in the village of Agua Preta, Bahia, Brazil. Periportal fibrosis was determined by ultrasound. Peripheral blood mononuclear cells were obtained by the Ficcol-Hypaque gradient and the expression of the surface markers CD28, CD69, CD25 and CTLA-4 on TCD4+ and TCD8+ cells were determined by flow cytometry. The frequency of TCD4+ cells was similar in patients with different degrees of periportal fibrosis, however, individuals with moderate to severe fibrosis showed a lower frequency of TCD8+ cells when compared to patients without fibrosis or incipient fibrosis. The frequency of TCD4+CD28+ cells was higher in patients with moderate to severe fibrosis (mean = 95.7%) when compared to those with incipient fibrosis (62%). The frequency of TCD4+ and TCD8+ cells expressing CD69 did not differ between groups. The frequency of CD4+CD25Neg cells was higher in patients with moderate to severe fibrosis, compared to the other groups, while the frequency of CD4+CD25High cells was lower in patients with moderate to severe fibrosis (1.0%) compared to those without fibrosis (1.8%) or with incipient fibrosis (1.75%; $p<0.05$). The frequency of TCD4+CTLA-4+ cells in individuals with moderate to severe fibrosis was also lower (0.41%) than in patients without fibrosis (0.8%) or with incipient fibrosis (1.6%; $p<0.05$). The low frequency of T cells with regulatory profile in schistosomiasis patients with periportal fibrosis may be associated to the high frequency of activated T cells and therefore leading to liver pathology in these patients.

428

GENETIC AND PHARMACOLOGICAL ANALYSIS OF TRANSIENT RECEPTOR POTENTIAL (TRP) CATION CHANNELS IN *SCHISTOSOMA MANSONI*

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Though schistosomiasis affects hundreds of millions worldwide, only a single antischistosomal drug (praziquantel) is available in most parts of the world, a potentially dangerous situation. Reports of praziquantel resistance lend particular urgency to the need for new therapeutics. Ion channels allow ions to flow by diffusion down the electrochemical gradient established across cell membranes, and are essential to normal functioning of the neuromusculature and other tissues. They are validated targets for drugs and toxins in a number of systems, and, indeed, the majority of current anthelmintics act on ion channels. However, only a very few of the ion channels expressed by schistosomes and other parasites have been analyzed for their potential as targets for new drugs. One set of channels with promise for exploitation is the transient receptor potential (TRP) channel superfamily. TRP channels are a highly diverse superfamily of cation channels that share the common feature of mediating transduction of sensory signals. TRP channels also appear to play roles in modulating immune responses and lifespan, and are being intensively investigated as drug targets for a variety of human conditions ranging from chronic pain to cancer. To date, however, the functions and pharmacological sensitivities of schistosome TRP channels have not been determined. We are using pharmacological, genetic, and molecular biological tools to better understand the roles these channels play in *Schistosoma mansoni* physiology and survival. For example, we find that agents that selectively activate or antagonize mammalian TRP channels have dramatic effects on motility of *S. mansoni* adults and schistosomules. We are also using RNA interference to suppress expression of these channels and help further establish their role and potential for targeting. These studies, in parallel with heterologous expression studies, should provide important information about schistosome sensory physiology as well as novel candidate drug targets.

429

ASSOCIATION BETWEEN CHANGES IN SOCIOECONOMIC STATUS, WATER CONTACT AND *SCHISTOSOMA MANSONI* INFECTION IN AN ENDEMIC AREA IN MINAS GERAIS STATE, BRAZIL

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We evaluated changes in *Schistosoma mansoni* prevalence, socioeconomic status and water contact behavior for a cohort of 357 individuals between 2001 and 2009 in an endemic rural area Minas Gerais State, Brazil. Parasitological surveys using three stool samples per survey and the Kato-Katz method were carried out in 2001 and 2009 and all positive individuals were treated with praziquantel. Information on socioeconomic status (SES) and frequency of water contact was collected for each individual during household surveys using questionnaires. We used several binary variables indicating changes in household possessions and motorized vehicles between 2001 and 2009 for each individual. A generalized linear model was used to assess the association between demographic variables, SES and water contact with *S. mansoni* prevalence in both models (infection and reinfection). We also used a Bayesian method of variable selection to identify the most significant factors associated with prevalence. *S. mansoni* prevalence before treatment (2001) was 59.0%, with a geometric mean egg count (epg) of 61.05. In 2009, prevalence was 26.8% and intensity of infection had significantly declined to 8.78 epg. There was a slight decrease in the number of individuals who had unsafe contacts between 2001(75.3%) and 2009(70.8%). The Bayesian selection model revealed that changes in type of water contact from safe to unsafe and persisting poverty (individuals who had not acquired durable goods or improved their houses between 2001 and 2009 were jointly relevant in describing the risk of *S. mansoni* infection during the 8-year study period. Based on these results we conclude that both SES and water contact factors can be predictive of changes in *S. mansoni* prevalence in longitudinal studies using Bayesian analysis.

430

PRESCHOOL AND SCHOOL CHILDREN MALNUTRION AND SCHISTOSOMIASIS IN A VILLAGE FROM BENGO PROVINCE (ANGOLA): PRELIMINARY RESULTS FROM AN INTERVENTION STUDY

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Malnutrition and anemia are two of the potential consequences of Schistosomiasis, soil transmitted helminths and malaria. Those conditions coexist as endemics and potential factors causing several clinical consequences in children and compromising not only their growth and development but also their academic performance levels. The prevention and control of this disease involve the preventive administration of Praziquantel, and Albendazol, improvements in fresh water supplies, healthcare and education. This work presents the first results of a broader project aiming to contrast the results of the effectiveness of a school based programs and those of a community based programs as well as between pre-school age children and school age children, in Bengo province, Angola. An intervention study in a village from Angola, of 95 preschool children (2-5 year olds), 115 school-aged children (6-15 year olds) and 185 adults (> 15 year olds) was conducted to evaluate malnutrition, anemia, malaria, schistosomiasis and geohelminths and treat all intervenient with Praziquantel and Albendazol. Results: Malnutrition was common among children. In preschool children 13.4% were under-weight and 55.2% stunting. In school children 16.6% were under weight and 22.7 stunting. Anemia (<11.0 mg /dL) was found in 65% of children. Urinary schistosomiases prevalence reached 67% of preschool children and 82 % of school-aged children. Geohelminth infections were common, affecting 32% of preschool children and 58% of school-aged children. In conclusion, we report the first results of the intervention study and the results obtained justify the implementation of the interventions for the control of these diseases and morbidities, namely malnutrition and anemia. During the present year the effectiveness of the intervention and a comparison with a school intervention in a different village will be conducted.

431

A CASE-CONTROL STUDY OF NOROVIRUS-INDUCED ACUTE DIARRHEA AMONG ARMY RECRUITS IN PERU

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Norovirus is the number one cause of acute gastroenteritis worldwide and a leading cause of diarrhea deaths in children under five years old. However, few data are available on the incidence of norovirus in adults in developing countries. We conducted active surveillance for acute diarrheal illness among army recruits at the Vargas Guerra Army Base in Iquitos, Peru, from October 2005 through July 2011. To determine the frequency

of norovirus-induced diarrhea in this population, we performed a nested case-control study on 200 randomly selected cases with acute diarrhea and 200 randomly selected asymptomatic controls. Stools were tested for norovirus by RT-PCR; bacteria using culture and RT-PCR for *Escherichia coli*; and parasites using direct microscopy. Uni- and multivariate analyses were performed using Stata statistical software. Norovirus was present in 14.1% of cases and 8.0% of controls, with genogroup GII in 71%, GI in 25%, and mixed GI/GII in 4%. Norovirus-bacterial co-infection was present in 30%, including Shigella flexneri (15.4%) and enterotoxigenic *Escherichia coli* (15.4%). Norovirus-parasite coinfection was noted in 62% of cases, including *Trichuris trichiura* (27%), *Giardia* species (27%), *Ascaris lumbricoides* (23%), and *Uncinaria stenocephala* (8%). After controlling for co-infections, any norovirus infection and norovirus GII infection were associated with diarrhea, with adjusted odds ratios (AOR) of 2.8 (95% CI: 1.3-6.0, P<0.01) and 3.6 (95% CI: 1.4-9.2, P<0.01), respectively. Norovirus GI infection was not associated with diarrhea (AOR: 1.4, 95% CI: 0.4-4.3, P=0.61). Although norovirus was a frequent cause of acute diarrhea in this population, its frequent presence in controls suggests that additional factors beyond simple presence/absence of infection play roles in the development of disease. These may include size of the inoculum, co-infections, previous infection and adaptive immunity, and genetic predisposition. Further investigation of the possible role of these co-factors is warranted to fully understand the epidemiology of norovirus disease.

432

THE STATUS AND CHALLENGES FOR THE CONTROL OF RABIES IN NEPAL

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A study was conducted in Nepal to know the status of rabies. Five years data (from 2005 to 2009) on human rabies information recorded at Epidemiology and Disease Control Division (EDCD) under the Department of Health Service and animal rabies information recorded at Veterinary Epidemiology Centre (VEC) as well as Central Veterinary Laboratory (CVL) under the Department of Livestock services were analyzed to know the status of Rabies in Nepal. There were 411 outbreaks and 750 animal deaths in 45 (out of 75) districts of country from 2005 to 2009. A total number of 48,703 animals were given post exposure treatment after bitten by suspected rabid animals. Rabies has been reported in dogs, cattle, buffaloes, sheep, goat, pigs and horses. Over the 5 years, 112 dogs, 19 buffaloes, 14 cattle, 11 goats, 2 cats, 2 mongooses, 1 rabbit, 1 mouse, 1 bat and 1 swine brain samples were submitted at CVL for laboratory confirmation. In total 164 clinical samples were tested out of which 113 (68.9%) were positive for rabies. Rabies has been confirmed in 59.82% (67/112) dog, 84.21% (16/19) buffalo, 78.57 (11/14) cattle, 72.72 (8/11) goat, 50% (1/2) mongoose and 100% (1/1) mice samples. The total numbers of rabid human (hydrophobia) cases admitted to hospital from 2005 to 2009 were 163 however all of these cases were diagnosed on the basis of clinical symptoms and history of dog bite. None of the human cases were confirmed on the basis of laboratory tests. In total 120,398 persons had received post exposure treatment in this period from 48 hospitals at free of cost after bitten by suspected rabid animals.

434

THE ROLE OF IKKβ IN VEEV REPLICATION

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Venezuelan equine encephalitis virus (VEEV) belongs to the genus Alphavirus, family Togaviridae. Currently no antivirals or therapeutics are available to treat VEEV infections. Our preliminary studies with small molecule inhibitors had indicated that the NFkB cascade, more specifically the IKK component, may play an important role in VEEV infection of human cells. We aimed to characterize the interaction of the host IKK complex with the virus and the consequence to viral multiplication. Initial findings indicated that at early time points of VEEV infection the NF-κB complex was activated when compared to UV-infected samples (as inferred by phosphorylation of p65 and IkBa). The upstream event contributing to phosphorylation of p65 is the activation of the IKK complex. Our previous studies with a different virus (Rift valley fever virus) had indicated that the IKKβ component underwent macromolecular reorganizatio to form a novel form of the complex that was unique only to infected cells. This prompted us to investigate if the IKK complex undergoes a comparable macromolecular re-organization in VEEV infection. Column fractionated mock and VEEV infected cell extracts indicated a macromolecular re-organization of IKKβ in VEEV infected cells, which resulted in formation of lower molecular weight complexes. Well-documented inhibitors of the IKK complex, BAY-11-7082, BAY-11-7085 and IKK2 compound IV, were employed to determine the effects of this inhibition on infectious viral titers. A decrease in infectious viral particles and viral RNA copies was observed with inhibitor treatment in VEEV infection. The efficacy of these inhibitors was also tested on the wild type strain of VEEV (TrD) in U87MGs and neuronal cells where are potent inhibition was observed. IKKβ over-expression studies increased TC-83 replication. In contrast, IKKβ-/- knockout studies demonstrated a reversed phenotype, where TC-83 replication was inhibited. Finally, *in vivo* studies demonstrated that inhibitor treatment of TC-83 infected mice increased in their mean survival time. Thus far our studies have revealed that the host IKKb protein may be critically involved in VEEV multiplication. Ongoing proteomic studies are aimed at determining the mechanism behind the alteration of the IKKb complex during VEEV infection.

435

LASSA FEVER OUTBREAK INVOLVING HEALTH CARE WORKERS IN TARABA STATE, NIGERIA; MARCH 2012

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Lassa fever is an acute, highly infectious viral haemorrhagic illness caused by Lassa fever virus - a single stranded, RNA virus belonging to the virus family Arenaviridae. The reservoir is Mastomys natalensis. The disease is endemic in West African sub region causing 300,000-500,000 infections annually, with about 500 deaths. In March, 2012, we investigated a reported outbreak of Lassa fever in Taraba State, Nigeria to confirm the outbreak, determine its extent, characterize the outbreak, institute public health actions and make appropriate recommendations. We reviewed hospital records and used IDSR standard case definition for Lassa fever to identify and line-list cases. A suspected case was defined as "any person with severe febrile illness not responsive to the usual causes of fever in the area with or without sore-throat and at least one of the following: bloody stools, vomiting blood, bleeding into the skin and unexplained bleeding from the nose, vagina or eyes". A standardized line-listing form was developed to capture socio-demographic and clinical information of the cases. Various exposure factors including age, gender, occupation and contact history were examined. A total of 35 cases were recorded. Nine of 35 cases were laboratory confirmed (25.7%). Altogether, 14 deaths were recorded giving a case fatality rate of 40%. Majority of the cases belonged to the age group 25-34 years (40%) with females constituting 51%. Most of the cases were healthcare workers (22.9%). The commonest presenting features were fever (85.7%), cough (28.6%), bleeding from orifices or into skin (25.7%) and headache (20%). The State's Epidemic Management Committee was found to be non-functional resulting in uncoordinated response to the outbreak. In addition, many exposure factors to Lassa fever such as over-crowding, drying of food items along high ways and bush burning were identified and there was low index of suspicion of Lassa fever among health care workers. There was a confirmed outbreak of Lassa fever in Taraba State mostly affecting healthcare workers. Community sensitization and sensitization of health care workers in Taraba

State on Lassa fever were carried out. It was recommended that the State should reactivate its moribund Emergency Management Committee, surveillance of Lassa fever should be strengthened and Public/Health care workers sensitization activities should be scaled up.

436

UNDERSTANDING THE DYNAMICS OF CIRCULATION, REASSORTMENT AND TRANSMISSION OF NGARI AND BUNYAMWERA VIRUSES IN NORTHERN KENYA

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Although genetic drift/reassortment seems to occur frequently in the Bunyaviridae family, the epidemiological consequences of these evolutionary events are poorly understood. Our objective was to understand the dynamics of circulation, reassortment and transmission of Bunyamwera and Ngari viruses in Kenya. We identified isolates of both viruses which were relatively conserved regardless of the species or region of isolation with the later clustering closest with the Ngari strain associated with the 1997-1998 hemorrhagic fever outbreaks in East Africa. *Anopheles* gambie was a more competent vector for Ngari than Bunyamwera virus possibly due to genetic reassortment. This has major implication in light of continued animal trade and travel especially into malaria endemic regions where *Anopheles gambiae* is more prevalent. Our results underscore the need to continually monitor emergent arboviral genotypes circulating within particular regions as well as vectors mediating these transmissions in order to preempt and prevent their adverse effects.

437

HEPATITIS E VIRUS INFECTION AT THE HUMAN ANIMAL INTERFACE, LAGOS NIGERIA

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Hepatitis E virus (HEV) is a leading cause of acute/chronic liver failure with higher severity in pregnant women. About one million human deaths are annually recorded globally due to viral hepatitis of which HEV contributes significantly. Pigs are considered natural reservoir host of HEV especially in intensive farms with close human-animal interaction coupled with poor sanitary practices. The zoonotic risk of HEV among occupationally exposed individuals in developing countries is of public health concern. However, data on disease burden for planning intervention in sub-Saharan Africa is lacking. This study describes the seroprevalence, of HEV at the human-animal interface in a pig estate in south western Nigeria. Cross sectional sampling of blood from randomly selected pigs and pig handlers of a multi-complex pig farm in Lagos, south western Nigeria was carried out. Seventy three human and 212 swine sera were collected. Sera were tested for anti-HEV IgG and IgM antibodies by two step double antigen sandwich ELISA using Hep.EV. According to manufacturer's protocol, two hundred and twelve (97%,) and 13 (17.8%) of swine and human sera were positive for HEV total antibodies respectively while 3 (1.4%) and 1 (1.3%) of swine and human sera were positive for HEV IgM respectively. 70% of pig handlers tested were women most of whom are within the reproductory age and one was pregnant. The high seropositivity observed and evidence of recent infection in pigs is a cause for concern. This is because there is a high risk of transmission of the virus to human contacts in a farm where over 80% of farms operate under poor biosecurity and waste management. This study provides information on HEV disease burden in pigs and pig handlers in the study area and emphasizes the zoonotic risk of HEV. In controlling HEV in this tropical region therefore, improvement in biosecurity practices including sanitation and proper waste disposal is strongly advocated. Further study on the genomic diversity of circulating HEV strains and the risk of feco-oral trasmission is recommended.

438

EFFECT OF ZINC SUPPLEMENTATION ON RESPONSE TO ORAL POLIO VACCINE IN INFANTS IN PAKISTAN: A RANDOMIZED CONTROLLED TRIAL

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Polio eradication remains a challenge in Pakistan and causes for the failure to eradicate polio are complex. Undernutrition and micronutrient deficiencies, especially zinc deficiency, have been identified as major public health problems in Pakistan and could potentially affect response to enteric vaccines. This study was undertaken to assess the impact of zinc supplementation among infants on immune response to oral polio vaccine (OPV). A double-blind, randomized placebo controlled trial was conducted in infants aged 0-14 days. Subjects were assigned to either treatment group receiving 10 mg zinc supplementation daily for 18 weeks or control group. Both groups received standard OPV doses at birth, at 6 weeks, 10 weeks and 14 weeks. Data was collected on demographic information and blood samples were collected to determine the antibody response to OPV and for micronutrient analysis. The prevalence of poliovirus seropositivity and seroconversion was examined using mixed effects logistic regression. Overall, 404 subjects were recruited; of which 303 were included in the crude analysis. At recruitment, seropositivity was already high for poliovirus (PV) serotype 1 (zinc: 89.7%; control: 90.4%) and PV2 (89.7%; 93.6%), with lower estimates for PV3 (69.9%; 65.6%). The proportion of seropositive subjects for PV3 at week 6 was higher for those in the zinc group [53.4 (45.3, 61.6)] than in the control [42.7 (34.9, 50.5)], albeit not significant (p=0.061). This trend was not observed at week 18. There were no differences between groups for PV1 or PV2 at either week 6 or week 18. In the multivariate logistic regression model, no association was found between seropositivity or seroconversion and zinc supplementation for either PV1, PV2, or PV3. In conclusion, zinc supplementation in Pakistani infants from two weeks of age was not associated with a significant impact on seroconversion to OPV.

439

MUTATIONS PRESENT IN WESTERN EQUINE ENCEPHALITIS VIRUS POSSIBLY ACCOUNT FOR REDUCTION IN HUMAN INCIDENCE

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Western equine encephalitis virus (WEEV) has caused epidemics that have resulted in the deaths of thousands of humans and equids over the past century. Interestingly, WEEV infection has decreased precipitously over the past fifty years, with the last documented clinical case observed in 1998. Depite this decline, the virus has still been found to be cirulating in nature until 2008 as evidenced by virus positive mosquito pools. The purpose of this study is to discover any mutaitons in WEEV that could account for this observed reduction in human incidence. To accomplish this, a robust collection of WEEV viruses were collected and whole genomes were sequenced. In order to determine what mutations define specific lineages, maximum parsimony, neighbor-joining, maximum likelihood, and Bayesian phylogenies were generated and amino acid mutations were traced using these phylogenetic trees. To elucidate possible changes in genetic diversity such as population bottlenecks or logistic population growth, we used Bayesian skyline reconstructions implemented in BEAST. Additionally, we estimated evolutionary rates and dates of divergence for the entire phylogeny and individual lineages. Several mutations were identified and their inferred dates of divergence appears to coincide with WEEV's observed reduction in human incidence. These mutations also define important lineages in the phylogeny. They are located in nsP3, nsP4, capsid, E1, and E2 genes. These results supply compelling evidence that WEEV's evolution over the past half century has resulted in a reduction of human virulence. These mutations will now be characterized and tested in order to determine if they do impart a phenotypic effect.

440

PERFORMANCE OF RISK EXPOSURE SCREENING QUESTIONS TO IDENTIFY NIPAH CASES ON ADMISSION IN SURVEILLANCE HOSPITALS IN BANGLADESH

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Drinking raw date palm sap and contact with Nipah case-patients are major pathways of transmission for Nipah encephalitis in Bangladesh. Ongoing hospital based meningo-encephalitis (ME) surveillance identifies Nipah cases through serological diagnosis; however, results often take weeks to obtain. Earlier detection of Nipah cases could improve our ability to prevent secondary transmission from patients to caregivers and healthcare workers through infection control. The objective of the study was to assess the performance of using reported risk exposures among ME cases in surveillance hospitals to detect Nipah cases on admission. From December 2012 to March 2013, study physicians in three Nipah surveillance hospitals assessed all ME cases for known risk exposures for Nipah infection by asking them or their caregivers if they had consumed raw or fermented date palm sap or had contact with any another ME case-patient in the month before onset of illness. We calculated the sensitivity, specificity, and positive predictive value (PPV) of these screening questions to identify Nipah cases by comparing them to results from IgM serology tests. We also calculated these performance measures for cases occurring during the peak Nipah incidence months in Bangladesh of January and February. From December 2012 to March 2013, we identified 360 ME cases and 328 (91%) had serum tested for anti-Nipah IgM. 19 (6%) cases had detectable IgM antibody against Nipah virus and 14 of these reported ≥1 known risk exposure, for a sensitivity of 74%. Among 309 ME cases who had no detectable IgM antibody, 264 had no known risk exposures, for a specificity of 85%. Among all ME cases, 59 (18%) had ≥1 known risk exposure, for a PPV of 24%. Among ME cases enrolled during January-February 2013, sensitivity was 93%, specificity was 83%, and PPV was 38%. In conclusion, screening for risk exposures on admission demonstrated high sensitivity and specificity for detecting Nipah cases, particularly during peak incidence months. On admission, screening criteria should be used to identify cases for whom more stringent infection control measures are necessary. Because the PPV of the screening questions was low, development of a rapid test kit for Nipah diagnosis would assist case detection. Considering limited diagnostic facility, low cost countrywide expanded surveillance focusing high risk exposures using screening criteria may identify more Nipah cases.

441

COMPLETE GENOMES OF INFLUENZA A SEASONAL H3N2, 2009 PANDEMIC H1N1 AND INFLUENZA B DETECTIONS BY NEXT GENERATION SEQUENCING

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Next generation sequencing (NGS) Illumina, MiSeq Platform was utilized to investigate the complete genome of six influenza virus isolates, which were positively identified using the CDC polymerase chain reaction (PCR) assay. The DNA library obtained from each virus was mixed and all were sequenced simultaneously. Total information of 2.6 Gbases was obtained from a 455±14 K/mm^2 density with 96.76% (8,571,655 / 8,950,724 clusters) of the clusters passing quality control (QC) filters. Approximately 93.7% of all sequences from Read1 and 83.5% from Read2 contained high quality sequences that were ≥Q30, a base calling QC score standard. The average error rate from base calling at the 100th cycle was measured as 0.20%±0.03% for both reads. Total six alignments analysis identified three Influenza A seasonal H3N2 strains (A/Brisbane/11/2010), one 2009 pandemic H1N1 (A/California/07/2009) strain and two Influenza B (B/Wisconsin/01/2010) strains. The nearly entire genomes of all six virus isolates yielded equal or greater than 600-fold sequence coverage depth. MiSeq Platform efficiently identified DNA library mixtures of Influenza A seasonal H3N2, 2009 pandemic H1N1 and Influenza B.

442

AN EPIDEMIC OF CHIKUNGUNYA IN NORTHWESTERN BANGLADESH IN 2011

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In November 2011, the primary healthcare (PHC) manager of Shibganj subdistrict of Chapainababganj District in northwest Bangladesh reported a cluster of patients with fever and joint pain or rash. We investigated the outbreak to identify the etiology, characterize the illness and estimate the attack rate. We defined a suspect case as a resident of Shibganj with fever, joint pain and/or rash with onset from September 1–December 31, 2011. To estimate attack rate, we conducted a household-based syndromic survey in 16 clusters using multistage cluster sampling. Trained field workers visited 80 households nearest to the local PHC clinic in each cluster to identify suspect cases. We invited one suspect case per household from the first 30 households per cluster to complete a structured questionnaire and provide a blood sample. We tested serum samples for immunoglobulin M (IgM) antibodies to Chikungunya virus (CHIKV) by capture enzyme-linked immunosorbent assay. Because of the time-lag between illness onset and laboratory investigations and the limited duration of IgM antibodies in serum, we used the number of suspect cases to estimate the attack rate. We surveyed 5,972 people from 1,283 households. Among these, 30% (1,769) met the suspect case definition; the median age was 28 (Interquartile range [IQR]: 15–42) years and 48% (856/1,769) were males. Among 480 suspect cases invited to participate in the sero-survey, 377 (79%) completed questionnaires; median age was 40 years and 64% (241/377) were females. 77% (377/480) provided a blood sample and 74% (278/377) had evidence of IgM antibodies to CHIKV. The outbreak lasted >3 months and peaked in early November. Predominant symptoms included fever (100%), joint pain (88%), debilitating weakness (55%), myalgias (44%), itching (33%) and rash (31%). The median duration of joint pain was 15 (IQR: 6-19) days and 7 (IQR: 5-13) days for debilitating weakness. 64% (239/377) of the suspect cases surveyed sought care from local PHC clinics. The 30% attack rate and the predominance of adult cases suggest that Shibganj's residents had little previous immunity to CHIKV. This was the second recognized epidemic in Chapainababganj, following a smaller outbreak in 2008, and underscores the continued risk of CHIKV epidemics in Bangladesh. Averting CHIKV in Bangladesh requires improved PHC-based surveillance to enhance outbreak detection, define transmission risk and allow early public health interventions.

443

EVALUATION OF HUMAN INFLUENZA VIRUS ISOLATION USING MDCK CELL CULTURE DURING SURVEILLANCE IN ASIA

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Laboratory influenza surveillance is critical to monitor influenza activity and to provide the epidemiological data needed for emerging pandemic preparedness and vaccine development. In addition, sample collection followed by viral propagation is required for more complete characterization. Since 2008, USAMC-AFRIMS has conducted influenza surveillance in Bhutan, Nepal, Philippines and Thailand. Respiratory samples are routinely identified and characterized by real-time RT-PCR (rRT-PCR), in which influenza viruses are further sub-typed. Virus isolation is done for both influenza rRT-PCR positive and negative samples using Madin-Darby canine kidney (MDCK) cell culture. From 2008 to 2012, 5,667 samples were randomly selected for virus isolation. While 49% of all respiratory samples tested by rRT-PCR in that group were positive for influenza virus (2,803 samples of 5,667), only in 54% (1,508 samples of 2,803) were we able to successfully isolate and identify the virus by immunofluorescence assay (IFA). The isolation rate varied among the different subtypes. Influenza A H1N1, A H3N2, A 2009 H1N1, and B were successfully isolated in 77, 62, 34, and 64% of the rRT-PCR positive specimens, respectively. The rate of successful influenza virus isolation strongly correlated with the rRT-PCR results. Respiratory samples with high viral content with low cycle -to-threshold value ($Ct \leq 20$), moderate viral content ($20 < Ct < 30$), and low viral content ($Ct \geq 30$) were successfully isolated at the rates of 84, 51 and 6%, respectively. The isolation rate varied from country to country, with samples collected from Bhutan showing the lowest rate (17%). The low isolation rate, as compared to rRT-PCR results, may be attributable to viral viability loss during collection and transport (highlighting the importance of handling and cold-chain monitoring) but also to a less than adequate cell substrate. These analyses provide useful information to consider when designing future influenza surveillance studies.

444

MOLECULAR CHARACTERIZATION OF ANTIVIRAL SUSCEPTIBILITY OF INFLUENZA A ISOLATES OBTAINED IN KENYA FROM 2008 TO 2011

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Presently, there are two main classes of antivirals in use which function by inhibiting specific steps within the virus replication cycle: M2 inhibitors block the uncoating of the virus through acidification of the interior of the virion. In neuraminidase inhibition, inhibitor molecules mimic NA's natural substrate and bind to the active site, preventing NA from cleaving host cell receptors and releasing new virus. The study characterized antiviral susceptibility of the 2008-2011 influenza A strains using known molecular markers in neuraminidase (NA) protein. In the 2008-2009, 2009-2010 and 2010-2011 influenza seasons, a total of 836 viruses were isolated. 344 (41%) were influenza A/H3N2, 144 (17%) seasonal influenza A/H1N1 and 348 (42%) belonged to the pandemic influenza A/ H1N1 strain. A total of 108 (13%) isolates were analyzed for susceptibility to NA inhibitors. In the year 2008, 33 influenza A/H3N2 and 11 seasonal influenza A/H1N1 were included in the genotypic characterization assay for neuraminidase inhibitor resistant mutations. Sequence assembly and alignment revealed absences of molecular markers of neuraminidase inhibitor drug resistance (Y275) in influenza A/H3N2. 64% (7) of the 2008 seasonal influenza A/ H1N1 isolates had resistant marker H275Y. 4 (36%) of the seasonal A/ H1N1 isolates, lacked the drug resistant marker depicting sensitivity to the class of drugs. Genetic analysis of the 48 pandemic influenza A/ H1N1 strains in 2009 showed that all were sensitive to oseltamivir through possession of histidine at position 274 of the neuraminidase protein sequence. The same pattern was duplicated in 2 of the pandemic influenza A/ H1N1 isolates analyzed in the year 2010. All the 2011, 14 isolates belonging to influenza A/H3N2 subtype lacked the H275Y substitution in the neuraminidase protein. Genotypic data obtained in this study demonstrate antiviral resistance in seasonal influenza A/H1N1 viruses isolated in Kenya in 2008-2009 through possession of H275Y (N1 numbering) marker in the neuraminidase protein.

445

INFLUENZA A VIRUS IN SWINE IN PERU

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Influenza viruses belong to the Orthomyxoviridae family and are divided into 3 types (A, B and C). Influenza A viruses are further classified into 17 HA and 9 NA subtypes that can infect a variety of hosts, including migratory waterfowl, resident birds, horses, swine, dogs, sea mammals, guinea pigs, bats and humans. Although different strains have evolved with each species, cross transmission of some influenza viruses between species is possible. Swine play a particularly important role as "mixing vessels" because they possess receptors recognizing both human and avian influenza A viruses. We sought to determine the prevalence of influenza A virus in swine raised in the Department of Lima and surrounding areas of the central coast of Peru. Surveillance was conducted at a central slaughterhouse in Lima. Upon arrival at the facility, animals were inspected for health status and kept in groups according to the seller for a maximum of 14 hours. Blood and nasal and tracheal swab specimens were collected at the time of slaughter. Serum was tested for IgG antibody to influenza A virus by ELISA (IDEXX Laboratories, Maine, USA) and the swab samples by rRT-PCR using the CDC Flu A assay to detect universal influenza A virus. PCR positive samples were further analyzed with subtype-specific primers. From December 2011 to May 2012, 963 adult pigs were sampled, with a prevalence of IgG antibody of 60% (573/958) and 4% (42/963) of animals positive by PCR. Subtype identification from the 42 PCR positive animals were 25 (60%) pandemic H1N1, 8 (19%) H3, 6 (14%) seasonal H1, and 4 (10%) un-subtypable, on which sequencing is presently underway. H1 pandemic/H3 coinfection was noted in 3 (7%) samples. Pigs in the study area are frequently infected with influenza A viruses, primarily human subtypes, providing ample opportunity for coinfections and reassortants.

446

SEVERITY OF ILLNESS AND CHRONIC MALNUTRITION IN CHILDREN WITH ROTAVIRUS-ASSOCIATED DIARRHEA IN GUATEMALA

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Rotavirus is the most important cause of severe diarrhea in young children in developing countries. Malnutrition impairs immune responses and may contribute to severity of diarrheal illness. We investigated the relationship between stunting, an indicator of chronic malnutrition, and severity of rotavirus-associated diarrhea in children <5 years in Guatemala. We enrolled children born after June 1st 2009, who were hospitalized

or treated in the emergency department for diarrhea in 4 hospitals in Guatemala, December 2012-April 2013. Stool samples were assayed for rotavirus using ELISA. Heights and weights were measured at presentation; growth stunting was defined as height for age Z-score (HAZ) <-2 (per WHO standards). Based on the 20-point Vesikari severity score, patients were categorized as having severe (≥11) vs. mild/moderate (<11) illness. Logistic regression models were constructed for calculating odds ratios (OR) for relationships between stunting and severity of illness among cases positive for rotavirus, adjusting for site (Guatemalan department), gender, age, history of breastfeeding, rotavirus vaccination, socioeconomic and sanitation variables. 231 children tested rotavirus positive. Among them, median age was 12 months (IQR 9-17), 72 (31%) met criteria for stunting, and 108 (47%) received at least one dose of the rotavirus vaccine, 209 (90%) had a history of being breastfed. 179 diarrhea cases (77%) were categorized as severe, of which 60 (34%) were stunted. We found a higher odds of severe diarrhea among children with stunting (aOR=2.6, 95%CI 0.9-7.7, p=0.07). Chronic malnutrition was associated with risk of developing severe rotavirus disease.

447

RIFT VALLEY FEVER SEROPREVALENCE IN COASTAL KENYA

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Rift Valley fever virus (RVFV) causes severe disease in both animals and humans that can result in significant economic and public health harm. The objective of this study was to measure the prevalence of RVFV exposure during 2009-2011 in six coastal Kenyan villages, and link seropositivity to demographics, socioeconomic status, mosquito density, and other risk factors. Demographic, household inventory and exposure questionnaires were administered to all participants. Sera were tested for anti-RVFV IgG via standard ELISA. Bivariate relationships for each potential predictor of RVFV seropositivity were assessed using a $\chi 2$ test. Multivariable logistic regression was used to further test predictor variables for association with seropositivity. Overall, 2,871 sera were tested; 51 (1.8%; 95%CI 1.3-2.3) were RVFV seropostive. Rates differed significantly among villages, with Jego having the highest rate (18/300; 6.0%; 95%CI 3.6-9.3) and Magadzoni having the lowest (0/248). Other village rates were as follows: Vuga 1.0% (8/835; 95%CI 0.4-1.9); Kinango 1.0% (5/524; 95%CI 0.3-2.2); Nganja 1.7% (7/404; 95%CI 0.1-3.5); and Milalani 2.3% (13/560; 95%CI 1.2-3.9). Adults were more likely to be seropositive than children (p<0.001), but there were no statistically significant differences between genders. Those who owned land were also more likely to be seropositive (p<0.001). There was not a significant correlation between livestock or *Culex* density and human seroprevalence. However, the two highest prevalence villages experienced periodic flooding during this time, and the highest prevalence village, Jego, was adjacent to a herding community that had exceptionally high livestock and cattle numbers. Overall, Rift Valley Fever exposure is low in coastal Kenya, although significant village level variation is present. As in other regions, adults are more likely to be exposed.

448

MOLECULAR CHARACTERIZATION OF GROUP C ORTHOBUNYAVIRUSES ISOLATED IN PERU

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Group C viruses are a complex of viruses in the genus *Orthobunyavirus*, family *Bunyaviridae*. These viruses are associated with human febrile disease in tropical and subtropical areas of South and Central America. While numerous group C orthobunyaviruses have been isolated from mosquitoes, animals, and humans, genetic analysis of these viruses is limited. Since the 1990s, we have conducted passive surveillance for febrile disease in Peru. During this time, 65 virus isolates from febrile patients in the northern and southern Peruvian Amazon were identified as group C orthobunyaviruses using an immunofluorecent assay. To further characterize these isolates, a 500bp region of the S segment was sequenced. Pairwise sequence analysis of the clinical isolates showed nucleotide identities ranging from 71% to 100% and deduced amino acid sequence identities ranging from 74% to 100%. For comparison, we sequenced prototype strains from the following group C antigenic groups: Caraparu virus (CARV), Murutucu virus (MURV), Oriboca (ORIV), Marituba (MTBV) and Apeu (APEUV). Sequence comparison of the clinical isolates with the prototype strains showed that 48 isolates had 85 - 88% nucleotide and 96 - 98% amino acid identity with CARV and 17 isolates had 82 - 86% nucleotide and 96 - 97% amino acid identity with MURV, ORIV, APEUV and MTBV. Based on phylogenetic analysis, sequences could be separated into two clades. One clade contained viruses from both the northern and southern Peruvian Amazon and the prototypical CARV strain, the other clade contained clinical isolates from the northern Peruvian Amazon and the prototypical ORIV, MURV, APEU, and MTBV strains. These results demonstrate the genetic relationships of group C viruses circulating in the Peruvian Amazon and the genetic divergence of group C viruses within the northern Amazon.

449

ANALYSIS OF BLOOD STAGE MALARIA IMMUNITY INDUCED BY CHEMICALLY ATTENUATED PARASITES

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The development of vaccines for malaria has focused on the induction of antibodies to parasite surface antigens, most of which are highly polymorphic. An alternate strategy has evolved from observations that low-density infections curtailed by drug treatment can induce antibody-independent immunity to different strains. To test this strategy as a vaccine approach we treated parasitized red blood cells from the rodent parasite, *Plasmodium chabaudi*, 98 % of which were at the early 'ring' stage, with seco-cyclopropylpyrroloindole analogs, which covalently bind parasite DNA, and administered these to mice without adjuvant. DNA from the vaccine could be detected in the blood for over 110 days although we have not been able to visualize intact parasites. A single vaccination induced profound immunity to different malaria parasite species. Immunity was mediated by CD4+ T cells and was dependent on the red cell membrane remaining intact. T cells from vaccinated mice responded *in vitro* to both homologous and heterologous strains. Activation of T cells was antigen-specific since adoptively transferred ovalbumin-specific (OTII) cells were not activated by vaccination. Vaccine-induced T cells expressed

activation markers within 5 days of vaccination and contained intracellular gamma-interferon. Immunity could not be transferred with antibodies from vaccine-protected mice. Immunity persisted for at least 6 months. The human parasite, *P. falciparum*, could also be attenuated and we are now undertaking a human Phase I trial using these parasites prepared in our GMP-compliant facility. We believe that this approach will have relevance to the development of other parasite vaccines.

450

HUMORAL RESPONSES AGAINST *PLASMODIUM FALCIPARUM* AFTER CHLOROQUINE PROPHYLAXIS AND SPOROZOITE (CPS) IMMUNIZATION AND THEIR CORRELATION WITH PROTECTION IN HUMAN VOLUNTEERS AND A NOVEL MURINE CPS-MODEL

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Malaria vaccines are urgently needed for better control and eventually elimination. One of the major hurdles in vaccine design and development is incomplete understanding of protective immunity. Protection against malaria challenge infection can be induced by repeatedly exposing healthy human volunteers to *Plasmodium falciparum* infected mosquito bites during a prophylactic course of chloroquine (Chemoprophylaxis and Spozoroites, CPS). This regime allows full exposure to the clinically silent liver-stage of infection during the immunization period, whilst limiting exposure to the pathogenic blood-stage parasite. Antimalarial antibody responses have long been known to contribute to protective immunity against the disease, so we wanted to understand the development of humoral responses during repeated malaria infection under drug cover. We investigated the kinetics and antigen-specificity of antibodies and memory B-cells (MBC) during immunization and their correlation with protection against challenge infection by performing standardized MBC ELISpot and ELISA on sequential peripheral blood mononuclear cell and plasma samples from 38 healthy Dutch adult volunteers enrolled in two clinical CPS-immunization trials. Antigen specificity was assessed using nine antigens representing the different life-cycle stages of the malaria parasite. We demonstrate, for the first time, that CPS-immunization induces MBCs and antibodies specific for liver- and cross-stage antigens, which are gradually acquired over the course of CPS immunization and boosted upon malaria challenge. Importantly, CPS-induced MBC responses are more stable and more efficiently boosted after challenge, as compared with plasma antibody titres. Levels of MBC and antibodies to CSP, LSA-1, AMA-1 and MSP-1, the most dominant humoral responses during malaria infection, correlate with the degree of parasite exposure during immunization, but are not predictive of protection. Therefore, to determine the role of B cells in protection to malaria challenge following CPS immunization, we developed a novel experimental mouse model that replicates our human clinical trials. We show that B cells are absolutely essential for protection to malaria challenge following CPS-immunization in mice. In the future this model will allow us to dissect protective antigens to support the rational development of novel malaria subunit vaccines.

451

NATURALLY ACQUIRED *PLASMODIUM FALCIPARUM* RH5-SPECIFIC IGG ANTIBODIES CORRELATE WITH PROTECTION FROM MALARIA

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Vaccine strategies targeting *Plasmodium falciparum* asexual blood stages, which cause the clinical manifestations of malaria, have yet to show protective efficacy in clinical trials. *P. falciparum* reticulocyte-binding protein homologue 5 (PfRH5), an essential merozoite protein involved in erythrocyte invasion, is an attractive candidate for a blood-stage vaccine. We investigated the association between naturally acquired PfRH5-specific IgG present before the malaria season in Mali and the prospective risk of malaria using time from first PCR-confirmed *P. falciparum* blood-stage infection to first malaria episode as the primary outcome. *P. falciparum* infections were detected by retrospective PCR analysis of dried blood spots which had been collected every 2 weeks for 7 months, and clinical malaria episodes were detected by weekly active surveillance and self-referral. Baseline IgG responses to PfRH5 and another blood-stage antigen *P. falciparum* apical membrane antigen 1 (PfAMA1) were determined by ELISA in 342 individuals aged 6 months to 25 years who began the study free of blood-stage *Plasmodium* infection. 284 individuals subsequently became infected with *P. falciparum* by PCR detection during the ensuing malaria season. A significant delay in median time from blood-stage infection to first malaria episode was observed among individuals with positive IgG responses to PfRH5 (n=48; 71 days; 95% CI, 21-229 days) compared to individuals with negative responses (n=236; 18 days, 95% CI, 14-26 days) by log-rank test (P=0.001). After adjustment for age, sickle cell trait, anemia, and PfAMA1 IgG levels using a Cox proportional hazards model, the protective effect of PfRH5-specific IgG on malaria risk remained (HR 0.62, 95% CI, 0.42–0.94, P=0.02). PfRH5 IgG levels did not associate with reduced parasite density or multiplicity of infection at the first malaria episode. We also assessed the ability of naturally acquired PfRH5-specific antibodies to neutralize parasite invasion of erythrocytes *in vitro* using growth inhibition assays. These findings provide the first evidence for the role of naturally acquired PfRH5-specific antibodies in clinical immunity to *P. falciparum* malaria. Our methodology for assessing malaria risk improves the ability to detect associations between immune responses to blood-stage infection and clinical protection and may prove useful for evaluating potential correlates of blood-stage immunity.

452

ANTIBODIES TO SCHIZONT EGRESS ANTIGEN-1 (PFSEA-1) BLOCK SCHIZONT EGRESS FROM *FALCIPARUM* INFECTED RBCS

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We discovered PfSEA-1 using a differential screening approach contrasting plasma from children who were resistant or susceptible to *falciparum* malaria. Antibodies to the immunorelevant region of PfSEA-1 (rPfSEA-1A) predict resistance to severe disease in two yr olds and vaccination with rPbSEA-1A protects mice from *Plasmodium berghei* ANKA challenge. To further characterize PfSEA-1, we performed growth inhibition assays (GIA) and immunolocalization studies. For GIA assays, parasites were synchronized, plated at 0.3-0.4% *parasitemia*, and cultured to obtain mature trophozoites. Mature trophozoites were cultured in the presence of anti-rPfSEA-1A or pre-immune mouse sera. Parasites were cultured for 24 hrs and ring stage parasites were enumerated. Anti-PfSEA-1A inhibited parasite growth by 58-74% across three parasite strains (3D7, W2 and D10) compared to controls (all $P < 0.009$). In confocal and immunogold EM studies, PfSEA-1 localized to the schizont/parasitophorous vacuole membrane, Maurer's clefts and the inner leaflet of the RBC membrane. This localization of PfSEA-1 was not consistent with a role in RBC invasion; rather it suggested a role in parasite egress from iRBCs. To determine the mechanism of growth inhibition we performed schizont arrest assays (SAA) using anti-rPfSEA-1A. For SAA, parasites were synchronized three times using sorbitol, plated at 3.5% *parasitemia*, and cultured to obtain early schizonts. Early schizonts were cultured in the presence of anti-rPfSEA-1A or pre-immune mouse sera. Schizonts were enumerated at 12 hrs post-treatment and the percent of schizonts arrested calculated. Anti-rPfSEA-1A dramatically inhibited schizont egress resulting in 4.3-6.8 fold higher proportion of schizonts across three parasite strains compared to controls (all $P < 0.009$). Our data support PfSEA-1 as a novel vaccine candidate for pediatric *falciparum* malaria. By blocking schizont egress, PfSEA-1 may synergize with vaccines targeting hepatocyte and red cell invasion.

453

HETEROLOGOUS PROTECTION AGAINST MALARIA IN A SUBSET OF VOLUNTEERS AFTER IMMUNIZATIONS WITH *PLASMODIUM FALCIPARUM* SPOROZOITES UNDER CHLOROQUINE PROPHYLAXIS

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We previously reported that long-lasting homologous protection against *falciparum* malaria can be induced in malaria naive subjects on chloroquine prophylaxis, by bites of *Plasmodium falciparum* infected mosquitoes using the ChemoProphylaxis and Sporozoites (CPS) protocol. In a clinical follow-up study we assessed whether CPS-immunized and protected subjects were protected against a heterologous strain. Twelve CPS-immunized subjects exposed to either 3x15, 3x10 or 3x5 NF54 - infected mosquitoes and protected against a homologous NF54 (West-Africa) together with five malaria-naïve controls were challenged by the bites of 5 mosquitoes infected with the heterologous *P. falciparum* clone NF135. C10 originating from Cambodia at 14 months after the last immunization. The primary outcome was time to parasitaemia after challenge infection assessed by microscopy and qPCR. Two out of 12 previously CPS-immunized and protected subjects against NF54 were fully protected against a heterologous NF135.C10 re-challenge of which one subject was previously immunized with 3x 15 mosquito bites and one subject with 3x10 mosquito bites. The remaining volunteers developed *parasitemia* at a significant later time point (median pre-patent period by qPCR 10.5 days) than the controls who all had a pre-patent period (qPCR) of 7 days. In conclusion, CPS immunization can induce heterologous protection against a geographically and genetically distinct *P. falciparum* NF135 clone at 14 months after the last immunization with NF54. The pre-patent period in partially protected subjects was significantly delayed. Unprotected subjects showed strong and short adverse reactions to heterologous challenge which may be immune-mediated in response to blood stages and is an indication to partial protection. These initial data provide a basis to further explore CPS-induced heterologous protection, critical for clinical development of whole sporozoite-based vaccines.

454

ANTIBODY PROFILES INDUCED BY CONTROLLED HUMAN MALARIA INFECTIONS IN HEALTHY VOLUNTEERS UNDER CHLOROQUINE PROPHYLAXIS

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Complete sterile protection to *Plasmodium falciparum* (Pf) infection can be experimentally induced by controlled human malaria infections in healthy volunteers under chloroquine prophylaxis, through immunization with sporozoites delivered by bites of infected mosquitoes (CPS protocol). To characterize the profile of induced antibody specificities in blood samples collected at preimmunization and pre-challenge, we used a proteome microarray containing 811 Pf antigens recognized by plasma from naturally exposed individuals from Africa and by volunteers immunized with irradiated sporozoites. CPS-immunized and protected individuals generated antibodies against 174 Pf antigens, whereas reactivity of mock-immunized controls was negligible. Predominant reactivity was found against the pre-erythrocytic antigens circumsporozoite protein (CSP) and liver stage antigen 1 (LSA1), and to a lesser extent against MSP2, MSP8, MSP10, ApiAP2, and the hypothetical PF07_0053 antigen. In comparison, specimens of semi-immune adults from western Kenya showed a different profile with emphasis on blood stage antigens and scarce reactivity against LSA1 and CSP. The uniformly elevated reactivity of LSA1 and CSP in all of the protected individuals in this study highlights the potential importance of these two pre-erythocytic antigens in the protective response induced by CPS immunization. These data provide insight and guidance to pre-erythrocytic vaccine development by elucidating antigen targets involved in protective immunity against sporozoite and liver stages, however, the array used for this study is enriched in blood stage antigens and the liver stage proteome is underrepresented. Due to the importance of liver stage immunity associated with CPS-induced protection we are in the process of completing the Pf 3D7 proteome microarray to get a more complete understanding of pre-erythocytic immunity induced by the CPS protocol. This next generation Pf proteome array will contain more than 9,000 features covering all 5,400 encoded proteins, included hundreds of pre-erythocytic antigens that have never before been probed.

455

EFFECTIVENESS OF INSECTICIDE-TREATED BEDNETS TO REDUCE THE RISK OF MALARIA IN CHILDREN IN AN AREA OF MALAWI WITH SIGNIFICANT PYRETHROID RESISTANCE

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Insecticide-treated bednets (ITNs) are the cornerstone of malaria control in sub-Saharan Africa, but ITN effectiveness may be compromised in areas of pyrethroid resistance. Between 2011 and 2013 in Machinga District, Malawi, WHO resistance assays with *Anopheles funestus*, the predominant malaria vector, found mortality at 24 hours to be 0-48% for deltamethrin and 72% for permethrin. We conducted a cross-sectional survey in March and April 2012, prior to the start of an observational cohort study, to calculate the protective effectiveness (PE) of ITNs among children who did and did not sleep under ITNs. All households in six rural villages of Machinga District were censused and children aged 6 to 59 months were invited to participate. At enrollment, ownership, use, age and condition of ITNs was assessed by caregiver verbal report, and children provided finger-prick blood samples for polymerase chain reaction (PCR) testing of malaria *parasitemia*. Out of 1,667 participants, 1,200 (72%) met the inclusion criteria and provided written consent for enrollment. A total of 443 (37%, 95% confidence interval [CI] 34-40%) children were parasitemic by PCR. ITNs were used by 516 (45%) children, untreated bednets (UTNs, defined as any ITN >36 months old) were used by 388 (34%) and 253 (22%) children reported not using any bednet the night before the survey. Holes were noted in the ITNs of 302 (59%) children and in the UTNs of 304 (78%) children (p<0.001). Using a log-binomial model controlling for child age, household wealth, maternal education and the number of bednets within a 300m radius of the child's household, and accounting for clustering by household, the PE of ITNs in reducing the risk of malaria compared to no bednets was 25% (95% CI 10-37%) and the PE of UTNs compared to no bednets was 30% (95% CI 14-42%). Despite moderate to high pyrethroid resistance in this area, ITNs were effective at reducing the risk of malaria *parasitemia* in children 6 to 59 months old compared to no bednets. UTNs, however, were equally effective at reducing the risk of *parasitemia* in this age group, raising questions as to the mechanism by which ITNs may be protecting children against malaria.

456

THE PREVALENCE, CLINICAL FEATURES AND OUTCOMES OF PUTATIVE HEMOGLOBINURIA AMONG EAST AFRICAN CHILDREN RECRUITED TO THE FEAST TRIAL WITH SEVERE FEBRILE ILLNESSES

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Blackwater fever (BWF) remains poorly described among African children. Even less is known in terms of geographical distribution, innate characteristics, immunological, and infectious causation of this syndrome in children. We describe Dark Urine Syndrome (DUS) in East African children to understand its distribution geographically, with and without malaria, and associated complications of severe anaemia, jaundice, renal impairment and their outcomes in the six FEAST trial sites in East African countries involved. The analysis of FEAST data was done using STATA version 11.0. Continuous variables among the DUS were compared with those in the controls using the student's t-test and by ANOVA, while categorical variables were analysed using X2 - test. P-values of <0.05 were considered statistically significant and included in the multivariate analysis for risk factors for dark urine. The median age of children with DUS was 24 months (IQR 13, 38) without male predominance. A majority of patients with DUS 318/394 (81.0) were from eastern Uganda, where malaria transmission is very high; 256/318 (80.5%) also had clinical jaundice. Quality-controlled slide and/or rapid diagnostic tests for *P. falciparum* infection was positive in only 147/300 (49.0%) of DUS patients. Severe anaemia (Hb <5g/dL) complicated 238/310 (77.0%) of DUS compared to 480/1480 (32.4%) in the non-DUS (ND) group P 5mmol/L) was predominant in DUS 204/309 (66.0%) vs. 560/1450 (38.6%) in ND group P 20mmol/L) was marked in DUS group 123/187 (65.8%) vs 140/847 (38.6%) in ND group P <0.001. Mortality was similar in the DUS (12.3%) and ND group (9.9%) P = 0211. DUS in East African children is a complex phenomenon without an apparent single aetiology or pathophysiology. The syndrome was not limited to malaria alone as previously thought and without a male predominance G6PD as a major cause was highly unlikely. These novel findings of a high risk of renal injury and DUS indicate substantial geographical differences in the spectrum of severe disease across East Africa.

457

PREGNANCY MALARIA AND INFANT SUSCEPTIBILITY TO CLINICAL MALARIA IN AN AREA OF SEASONAL, INTENSE MALARIA TRANSMISSION

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Malaria in pregnancy is associated with poor pregnancy outcomes. In a previous study in Tanzania where transmission is perennial and intense, we observed that placental malaria modifies the susceptibility of infants to malaria infection, depending on the mother's parity. To assess this relationship in Mali where transmission is highly seasonal, pregnant women in Ouelesssebougou were enrolled and followed up to delivery, and their singleton children born between January 2011 and November 2012 were followed up to December 31st, 2012. Cox proportional hazards model was used to compare risk of clinical malaria episodes in the offspring, adjusted for potential confounders such as parity, use of insecticide impregnated nets (ITN), hemoglobin type, location and season. A total of 425 mother-offspring pairs were analyzed. Compared to other infants, infants born from mothers who had pregnancy malaria were 58% more likely to experience clinical malaria at a younger age (adjusted hazard ratio [AHR] = 1.58 (95% CI, 1.17 - 2.13). Although malaria in pregnancy is more frequent during first pregnancy, the risk of clinical malaria in firstborn children was lower compared to other children (AHR = 0.65 (95% CI 0.45 - 0.93). The risk of the clinical malaria was also lower with regular use of ITN (AHR = 0.73 (95% CI 0.54 - 1.00) and in infants with hemoglobin S (AHR = 0.54, (95% CI 0.29- 0.98). In conclusion, malaria in pregnancy is associated with increased risk of the clinical malaria in the offspring. First pregnancy, hemoglobin S and regular use of ITN reduce the risk of clinical malaria in offpring.

458

ESTIMATES OF THE RISK OF PLACENTAL INFECTION AND BURDEN OF LOW BIRTHWEIGHT ATTRIBUTABLE TO *PLASMODIUM FALCIPARUM* MALARIA IN AFRICA IN 2010

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Plasmodium falciparum infection during pregnancy leads to adverse outcomes including low birthweight (LBW). Women acquire immunity to malaria in pregnancy over consecutive pregnancies and are most susceptible during their first pregnancy. Using a model of the parity-dependent immunity acquisition and estimates of the geographical distribution of fertility and P.*falciparum* transmission, we estimate the number of women who could be expected to experience placental infection in the absence of pregnancy-specific interventions in Africa. By fitting our model to patterns of excess LBW risk in women experiencing placental infection in Kilifi, Kenya and Ifakara, Tanzania, we then estimate the burden of malaria-attributable LBW. Without pregnancy-specific protection we estimate that, in Africa in 2010, 11.4 (95% CrI 10.7-12.1) million pregnancies would have experienced placental infection at some stage of gestation, accounting for 41% of the total 27.6 million live-births. Combining this with our estimated relationship between placental infection and LBW, we found the potential LBW burden due to placental malaria was 900,000 (95% CrI 530,000-1,240,000) LBW deliveries per-year. The time at which the placenta becomes susceptible to infection, around the end of the first trimester, is a key period when we estimate 65% (95% CrI 61%-70%) of the potentially infected pregnancies first experience infection. Primigravidae experience a disproportionate 39% (95% CrI 33%-46%) of the total potential placental malaria-attributable LBW burden. These are the only contemporary estimates of the distribution of risk and associated LBW burden of malaria in pregnancy in Africa. They suggest that the risk of placental infection across Africa in unprotected women remains large. Prevention of malaria pre-conception or very early in pregnancy is predicted to have a major impact upon reducing LBW, particularly in primigravidae. Lifetime risk of LBW changes gradually with transmission, highlighting the need to maintain protection as transmission falls and the incremental benefit of malaria elimination.

459

EFFECTS OF RESTRICTING ARTEMISININ-BASED COMBINATION THERAPY TO TEST POSITIVE MALARIA IN A HIGH-TRANSMISSION SETTING IN GHANA: A CLUSTER-RANDOMIZED TRIAL

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The policy of test and treat malaria is replacing presumptive treatment in many endemic countries. There is however very limited data on the effect of test and treat policy on the incidence of malaria, anaemia and severe febrile illness in high-transmission settings. We conducted a cluster randomized trial to compare the effect of test and treat versus presumptive treatment in a high transmission setting in Ghana. Thirty two health centres were randomly allocated to test treat (TT) or presumptive treatment (PT) arms. Children aged 2-24months living around health centre were enrolled and the incidence of malaria, anaemia and severe febrile illness were measured by passive and active surveillance over 24 months. A total of 3046 children were enrolled in 32 health centres. The incidence of malaria in the TT arm was 696/1000 pyrs (95% CI 557, 870) and 800/1000 pyrs (700, 914) in the PT arm (P=0.56). The incidence of first or only episode of malaria after the first episode of febrile illness was 558/1000 pyrs (95% CI 434, 718) in the TT arm and 673/1000 pyrs (544, 833) in the PT arm (p=0.34); the incidence of anaemia was 82/1000 (63, 107) versus 92/1000 (71, 119) (P=0.61); incidence of severe febrile illness was 129/1000 (82, 210) versus 151/1000 (95, 250)(P=0.84). Children in the PT arm were more likely to be prescribed ACT than children in the TT arm (81.6% versus 72.1%; P=0.01). Restricting the use of artemisinin combination therapy to test positive malaria is appropriate even in high transmission settings.

460

SURVEILLANCE OF VECTOR POPULATIONS AND MALARIA TRANSMISSION DURING THE 2009/10 EL NINO EVENT IN THE WESTERN KENYA HIGHLANDS: OPPORTUNITIES FOR EARLY DETECTION OF MALARIA HYPER-TRANSMISSION

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Vector control in western Kenya highlands has resulted in a significant reduction of malaria transmission and a change in the vectorial system. Surveillance and monitoring is an important component of early transmission risk identification and management. However, below certain disease transmission thresholds, traditional tools for surveillance such as entomological inoculation rates may become insensitive. This study was undertaken toexamine the possibility of using a rapid diagnostic kit for the detection of anti-malaria immune markers circum-sporozoite protein antibodies and merozoite surface protein antibodies as an early indicator of transmission change. Indoor resting female adult malaria vectors were collected in four selected villages in U-shaped (Iguhu and Emutete) and the V-shaped valleys (Marani and Fort Ternan) for eight months. *Anopheles gambiae* complex was identified by PCR. Blood samples were collected from children 6-15 years old and exposure to malaria was tested using a circum-sporozoite protein and merozoite surface protein immunchromatographic rapid diagnostic test kit. Sporozoite ELISA was conducted to detect circum-sporozoite protein, later used for estimation of entomological inoculation rates. An upsurge in antibody levels was first observed in October 2009 in all the study sites while *Plasmodium falciparum* sporozoites were first observed in December 2009 at Iguhu and February 2010 at Emutete. Despite the upsurge in Marani and Fort Ternan no sporozoites were detected throughout the study period. The antibody-based assay was more sensitive and had much earlier transmission detection ability than the sporozoite-based assay. The proportion of An. *arabiensis* among *An. gambiae s.l.* ranged from 2.9-66.7% indicating a rearrangement of the sibling species of the *An. gambiae* s.l complex. In conclusion, the rapid diagnostic kit with molecular markers should be used with other malaria surveillance tools such as the climate based model in order for it to be efficient in the U shaped valleys which were the hotspots for malaria transmission.

461

MAJOR BURDEN OF SEVERE ANEMIA FROM NON-*FALCIPARUM* MALARIA SPECIES

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The burden of anemia attributable to the non-*falciparum* malarias in regions with *Plasmodium* co-endemicity is poorly documented. We

compared the hematological impact of all endemic *Plasmodium* species in southern Papua, Indonesia. Prospectively collected clinical and laboratory data were linked for all patients presenting to the main referral hospital over a 5 year period. Of 497,983 patients presenting to hospital (446,543 outpatients and 51,440 inpatients), a total of 115,972 (23.3%) were associated with a hemoglobin measurement. Of these 37,249 (32.1%) were infected with *Plasmodium* (*P. falciparum* n=23,716 [63.7%], *P. vivax* n=9,791 [26.3%], mixed infection n=2,931 [7.9%] and *P. malariae* n=790 [2.1%]). Patients with *P. malariae* had the greatest mean reduction in hemoglobin (-1.52 g/dL, 95% confidence interval [CI] -1.70, -1.35 g/dL) followed by those with mixed (-1.47 g/dL, 95%CI -1.58, -1.37 g/dL), *P. falciparum* (-0.91 g/dL, 95%CI -0.95, -0.87 g/dL) and *P. vivax* infections (-0.87 g/dL, 95%CI -0.93, -0.81 g/dL); p for all comparisons <0.001). Severe anaemia (Hb<5 g/dL) was present in 5896 (5.1%) patients, and was associated with a significant increased risk of death (OR = 4.77 [95%CI 4.23-5.36], p<0.001). Patients with mixed infection were at greatest risk of SMA (Adjusted Odds Ratio [AOR] 4.11, 95%CI 3.64,4.64; AORs for *P. falciparum*, *P. vivax* and *P. malariae* were 2.43 (95%CI 2.27,2.61) 2.43 (95%CI 2.23,2.66) and 2.74 (95%CI 2.12,3.53) respectively, p for all comparisons <0.001). *P. vivax* infection was the most common cause of malaria in the first year of life and accounted for 23.4% (95%CI 19.6,27.1%) of severe anemia in this age group. Overall, 9.7% (95%CI 8.8,10.6%) of severe anemia was attributable to non-*falciparum* infections compared with 11.7% (95%CI 10.6,12.7%) for *P. falciparum* monoinfections. In Papua, a resource poor setting, *P. vivax* is the dominant cause of SMA in infancy and mixed *P. vivax*/*P. falciparum* infection is associated with substantially greater hematological impairment than either species alone. These findings highlight the public health importance of integrated genus-wide malaria control strategies in areas of *Plasmodium* co-endemicity.

462

FEBRILE ILLNESS AND PRO-INFLAMMATORY CYTOKINES IN THE FIRST YEAR OF LIFE PREDICT IMPAIRED CHILD DEVELOPMENT IN BANGLADESHI INFANTS LIVING IN POVERTY

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An estimated one-third of children in low-income and middle-income countries fail to meet their full developmental potentials. The first year of life is a period of critical and rapid brain development and is also when most of the morbidity and mortality from infection is suffered. Recurrent infection results in stunted growth and stunting is a known predictor of cognitive impairment. It is, however, unknown whether infection has an independent role in cognitive development through mechanisms such as chronic inflammation. Studies in preterm infants have linked elevated levels of inflammation-related proteins near the time of birth to cognitive dysfunctions years later. We, therefore, hypothesized that clinical and biomarkers of inflammation in the first year of life can predict cognitive, language, and motor impairments in children living in an urban slum in Bangladesh. A cohort of 398 children in Dhaka, Bangladesh was observed from birth until 30 months of age. Febrile illness was used as a clinical marker of inflammation and elevated concentrations of inflammation-related cytokines (IL-1β, IL-6, TNF-α, IL-4, IL-10) in 6 month sera were used as biomarkers of inflammation. Psychologists assessed cognitive, language, and motor development using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) at 12 months, 24 months, and 30 months of age. Associations between febrile illness and elevated cytokine levels with developmental outcomes were evaluated using linear mixed models. Cognitive, language, and motor scores all declined significantly over time (all $p < 0.0001$). Duration of febrile illness during the first year of life was associated with impairments in language and motor development ($p = 0.003$ and 0.0003). Elevated levels of the proinflammatory cytokines IL-1β and IL-6 were significantly associated with impairments in motor development, while elevated levels of the immunomodulatory cytokine IL-4 were associated with higher cognitive scores (all $p < 0.05$). Our work has identified immune correlates of developmental outcomes in children, which could serve as both prognostic markers as well as potential immunological targets for interventions to prevent developmental impairment in at-risk children.

463

ALCOHOL, SOCIAL INSTABILITY, AND DEFAULT FROM MULTIDRUG RESISTANT TUBERCULOSIS TREATMENT IN RURAL SOUTH AFRICA

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Default from multidrug-resistant tuberculosis (MDR TB) treatment remains a major barrier to cure. As TB treatment programs transition to managing MDR TB entirely in the outpatient setting, the patients at highest risk for default during out-of-hospital treatment must be identified so that efforts to increase adherence can focus on them. In this study, we retrospectively analyzed a cohort of 225 patients who initiated MDR TB treatment at a rural TB hospital in the Western Cape Province, South Africa, from 2007 through 2010. We performed multivariable proportional hazards regression analysis to identify baseline risk factors for default, and we compared default rates, stratified by key risk factors, across relevant time intervals, seeking to understand high-risk time periods for default in relation to the hospitalization period. Fifty percent of the patients in this cohort were cured or completed treatment, 27% defaulted, 14% died, 5% failed treatment, and 4% transferred out. 63% of patients reported recent alcohol use. Younger age (hazard ratio [HR]=1.03 [CI 1.01-1.06]/year), informal housing (HR=2.8 [1.5-5.0]), lack of steady employment (HR=2.6 [1.1-5.9]), Cape-Coloured ethnicity (HR=2.3 [1.1-5.0]), and alcohol use (HR=2.1 [1.1-4.0]) were associated with default ($P<0.05$). Defaults occurred throughout the first 18 months of the two-year treatment course but were especially frequent among alcohol users after discharge from the initial four-to-five-month in-hospital phase of treatment, with the highest default rates occurring among alcohol users in the first two months after hospital discharge. We conclude that default is a major barrier to cure of MDR TB in this rural farming population and that interventions designed to increase adherence among young, economically-unstable patients and alcohol users could potentially increase cure rates. In particular, alcohol abusers need extra support during outpatient treatment in order to remain adherent.

464

UTILITY AND CONCORDANCE OF TST, IGRA, AND IP-10 IN DETECTING NEW *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN HOUSEHOLD CONTACTS FROM VITÓRIA, BRAZIL

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Tuberculosis (TB) continues to be a major cause of morbidity and mortality worldwide, most significantly in resource-limited settings. Although most individuals enter a state of clinical latency upon infection with *Mycobacterium tuberculosis* (MTB), about 5% develop progressive TB within two years of infection. To meaningfully interrupt disease transmission, we need more accurate and efficient identification of individuals at greatest risk of progression, i.e. those newly infected. To evaluate the use of tuberculin skin testing (TST) and the interferon-γ (IFNγ) release assay (IGRA) in those recently exposed to MTB, we enrolled 146 household contacts (HHC) of TB cases in Vitória, Brazil. TST was performed post-exposure and if negative, again at 8-12 weeks. HHC were classified as initially TST-positive (TSTpos), persistently TST-negative (TSTneg), or TST-converters (TSTc), the latter representative of new infection. Additional testing at 8-12 weeks included IGRA and measurement of IFNγ inducible protein-10 (IP-10), a molecule downstream of IFNγ signaling hypothesized to improve sensitivity of IGRA testing. There was generally poor agreement between TST and IGRA results (kappa=0.35). Of the 24/70 individuals who converted their TST from negative to positive, only 33.3% tested IGRA-positive. This is compared to 65.3% of TSTpos HHC who tested IGRA-positive, suggesting there may be a delay in IGRA conversion beyond 8-12 weeks post-exposure. IGRA testing differentiated TSTpos from TSTneg ($p<0.001$) but there was no difference between TSTneg and TSTc ($p=0.25$). Results were similar for IP-10. Lowering the cutpoint for a positive IGRA from 0.35 to 0.15 IU/ml increased the sensitivity of detecting TSTc from 33.3% to 45.8% although decreased specificity from 84.8% to 78.3%. Similarly, measuring IP-10 improved sensitivity for detecting TSTc and TSTpos, however, compromised specificity. Our results suggest that using IGRA and/or IP-10 for diagnosing new MTB infection may be limited by a delayed conversion to test positivity.

465

ASSOCIATIONS BETWEEN INFLUENZA ACTIVITY IN KENYA AND TEMPERATURE, RAINFALL, SPECIFIC HUMIDITY AND SOLAR RADIATION, 2010-2011

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Temperature and humidity are thought to be associated to seasonal influenza circulation in the temperate regions, but this relationship is less clear in the tropics. We analyzed the association between meteorological parameters and influenza frequency in 10 sentinel surveillance sites in Kenya (8 public hospitals and 2 refugee camps) that include tropical, arid and temperate-like climate zones. We obtained the weekly proportion of samples testing positive for influenza from persons visiting outpatient clinics for Influenza-like Illness (ILI) and those hospitalized with Severe Acute Respiratory Illness (SARI) during 2010 to 2011. Night Land Surface Temperature (a proxy for minimum air temperature), rainfall, specific humidity (SH) and solar radiation were retrieved from NASA's satellite and land model. For each site, we modeled the proportion of samples testing positive using binomial regression with the meteorological parameters entered via backward selection. We found SH was significantly associated ($p<.05$) either positively or negatively in all sites (negative in 8 sites, positive in 2 sites). The 2 sites with positive association were Mombasa (coastal region) and Dadaab (refugee camp), which had higher average SH (16 and 14 g/kg, respectively) compare to other sites (average 12 g/kg). In addition, influenza positivity was also significantly associated with: increasing rainfall and solar radiation in Mombasa; decreasing solar radiation in Kakuma Camp; decreasing temperature in Kakamega district. When the regression was applied to all areas at once, influenza positivity was significantly associated ($p<.05$) only with decreasing specific humidity. In conclusion, our findings underscore the link between influenza positivity and SH across Kenya's diverse climatic zones. The positive relationship with SH in the coastal Mombasa is consistent with our previous findings in other coastal countries such as El Salvador and Sri Lanka. Given recent literature that also suggests an interaction between rainfall and SH to predict influenza, further analyses in Kenya will also investigate this.

466

INCIDENCE AND CHARACTERISTICS OF PATIENTS HOSPITALIZED WITH INFLUENZA INFECTIONS FROM A RESPIRATORY DISEASE SURVEILLANCE SYSTEM, GUATEMALA, 2008-2012

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Influenza is a major cause of respiratory illness affecting 5-15% persons worldwide, and resulting in 3-5 million severe cases and 250,000-500,000 deaths each year. Influenza-related hospitalizations cause substantial health and financial burden. We used an active facility-based surveillance system for acute respiratory disease in three hospitals in Guatemala to estimate the incidence of laboratory-confirmed hospitalized influenza cases (adjusted for healthcare seeking behaviors), characterize the cases, and identify risk factors associated with admission to the intensive care unit (ICU) or death. Laboratory confirmation was by real-time reverse transcriptase polymerase chain reaction. Multivariable logistic regression was used to identify risk factors for ICU admission or death, adjusted for age and sex. From May 2008 to July 2012 we identified 446 hospitalized influenza patients, 362 (81%) had influenza A and 84 (18%) had influenza B. The median age of case-patients was 2.4 years (interquartile range: 0.7-32.3). Median length of hospitalization was 5 days (range: 0-77). Eighty (17.9%) were admitted to the ICU, 28 (6.2%) died; overall, 88 (19.7%) experienced either ICU admission or death. Children aged <6 months comprised 19% of cases, 22% of those admitted to the ICU, and 7% of the deaths. Other deaths occurred in 11 (6%) children aged 7-60 months, 6 (6%) persons aged 5-50 years, and 9 (11%) patients aged ≥50 years. Women of child-bearing age comprised 6% of cases (2 admitted to ICU; 1 death). In multivariable analyses, being from Santa Rosa surveillance site, being of Amerindian ethnicity, and having radiologically-confirmed pneumonia were independently associated with ICU admission or death. The annual incidence of hospitalized laboratory-confirmed influenza in Santa Rosa and Quetzaltenango was 19.7/100,000 overall and 85.4/100,000 for children aged <5 years. Influenza is a major contributor of hospitalization due to respiratory diseases in Guatemala. Our findings warrant further investigations to explore the utility of enhanced influenza prevention strategies.

467

USE OF THE FILMARRAY RESPIRATORY PANEL IN A RESOURCE-LIMITED POPULATION BASED COHORT-SETTING TO DETERMINE THE ETIOLOGY OF INFLUENZA-LIKE ILLNESS

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Influenza-like illness (ILI) can be caused by many pathogens that cannot be distinguished clinically. Conventional lab methods can be time consuming, labor intensive and only identify a single pathogen. The lack of an identifiable etiology can lead to empirical treatment of ILI due to lack of timely information. The FilmArray Respiratory Panel (RP) addresses many of these issues by providing an on-site, rapid testing methodology for 18 bacterial or viral respiratory pathogens. In August 2012, we implemented the FilmArray RP at our Puerto Maldonado, Peru satellite lab for use in an active household ILI surveillance study. During 33 weeks of surveillance, 413 ILI episodes were identified (7.9 episodes/1000 person-week of follow-up). The median age of ILI cases was 5 years old (IQR 12.7; SD 15.9). Samples were collected from 93.2% (385/413) of the ILI cases. The FilmArray RP identified at least one respiratory pathogen in 76.1% (271/356) of samples. A single pathogen was identified in 65.7% (234/356) of samples and multiple pathogens were identified in 10.4% (37/356) of samples. Among samples with a single etiology identified, human rhinovirus/enterovirus was identified in 29.9% (70/234) of samples; followed by influenza A H3, 23.1% (54/234); influenza A H1-2009, 11.5% (27/234); human metapneumovirus, 7.7% (18/234); respiratory syncytial virus (5.6%); and coronavirus OC43, 4.7% (11/234). The most common co-infection was human rhinovirus/enterovirus + parainfluenza virus 3 (29.4%,10/34). Three ILI (0.7%) required hospital admission; the etiologies identified were influenza A H1-2009, human metapneumovirus, and an influenza A H3 + human metapenumovirus co-infection. Numerous pathogens cause an ILI syndrome and influenza A was the predominant pathogen in our study. However, the FilmArray RP allowed us to determine the breadth of pathogens causing ILI and provide the opportunity for additional studies. Furthermore, on-site use of the FilmArray reduced time to results and reduced labor, shipping and lab testing costs.

468

GLOBAL POPULATION STRUCTURE AND GENETIC EVIDENCE OF SELECTION OF SULFA RESISTANCE IN *PNEUMOCYSTIS JIROVECII*

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Pneumocystis jirovecii is a commensal respiratory pathogen that causes pneumonia (PcP) in immunosuppressed patients, principally those with advanced HIV infection. Because *P. jirovecii* cannot be cultured, it has proven difficult to study, and little is known about its global population structure and drug resistance profile. Using sequence from a *P. jirovecii* draft genome, we developed assays for 8 putatively neutral microsatellite *loci* and 1 locus adjacent to the dihydropteroate synthase (*dhps*) gene, in which mutations are thought to be associated with exposure to sulfa drugs and potential sulfa drug resistance. Using these assays, we genotyped isolates from Uganda (n=13), the United States (n=26) and Spain (n=29), investigated their population structure, and quantified evidence for selective pressure on mutant *dhps* haplotypes. In all three populations, the 8 neutral markers demonstrated high levels of heterozygosity (H_e = 0.586 - 0.841). Although Nei's genetic distance indicated an overall genetic relatedness between populations (Uganda-Spain: 0.152; Uganda-US: 0.068; US-Spain: 0.034), when analyzed by an ecological clustering algorithm specimens from the three geographical populations demonstrated significant divergence of populations by continent. Notably, the single *dhps*-linked microsatellite marker demonstrated substantially lower diversity in isolates bearing mutant *dhps* haplotypes (H_e=0.305) compared with those bearing wildtype *dhps* haplotypes (H_e=0.682). This reduction in allelic diversity suggests that mutant *dhps* haplotypes have evolved and spread owing to selective pressure, most likely the exposure to sulfa drugs that are used for prevention and treatment of PcP as well as a wide variety of other bacterial and parasitic infections. The microsatellite-based multilocus genotyping method presented here represents the first genotyping tool to use selectively neutral *loci* for *P. jirovecii*, and will enable molecular-epidemiological investigations into *Pneumocystis* population structure, transmission, carriage, and drug resistance in human populations.

469

THE ORGANIZATION AND EVOLUTION OF PIRNA CLUSTERS IN *ANOPHELES GAMBIAE*

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The Piwi-interacting RNA (piRNA) pathway is an important mechanism in the defense against transposable element (TE) mobilization in many species including *Drosophila melanogaster* (the fruit fly), *Aedes aegypti* (the Yellow Fever mosquito), and *Mus musculus* (the mouse). In *Drosophila*, it has been shown that clusters of piRNA produce transcripts that, when interacting with PIWI proteins, create a complex that can recognize and silence TEs in the germ-line. In *Aedes* mosquitoes and the mouse, piRNA clusters appear to involve a higher number of protein coding genes. The aims of our study were 1) to see if the piRNA mechanism in *Anopheles gambiae* is more closely related to the *Drosophila* pathway or to the *Aedes* pathway and 2) to test if incipient species of *An. gambiae* differ in the structure of piRNA clusters. Here, we show the results of piRNA sequencing of the M and S forms of *Anopheles gambiae s.s.* A database of uniquely mapping piRNAs has been developed and subsequently mapped to the chromosomal map developed for the PEST reference genome, a mixture of genomic sequences of the M and S forms. We have also identified clusters of piRNAs based on the mapping performed on the PEST genome. The largest clusters of piRNAs were, as expected, found primarily in high TE-content areas- the intercalary and peri-centromeric heterochromatin. The top 15 clusters identified in *Anopheles gambiae* could potentially produce ~74% of the total number of piRNAs. Our results demonstrate that piRNAs, much like in *Aedes* and *Drosophila*, are present and active in Anopheline mosquitoes, but differ between species. Divergence in piRNA sequences and cluster composition suggests that the defense mechanism in the two species has begun to rapidly evolve to protect its respective genome against novel TE invaders that are not accessible to both populations. Cluster locations and content also suggest that the piRNA pathway in Anopheline mosquitoes is an intermediary between the *Drosophila* and *Aedes* pathways, as the piRNA clusters appear to contain a higher quantity of TEs than is seen in *Aedes*. Our data suggests that although speciation between the M and S forms is recent, there are differences in the TE vestiges that make up the clusters, as well as the piRNA sequences that are both present and absent when mapped to their respective genomes.

470

CRE-RECOMBINASE MEDIATED CASSETTE EXCHANGE IN *AEDES AEGYPTI*

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Approaches to generate transgenic mosquitoes rely on the random incorporation of a transposable element or site-specific integration utilizing a previously characterized targeting site. To date, the only site-specific technique currently used in disease-vector mosquitoes is the phiC31 system. Efficiency of recombination using this system varies extensively by target site and successful recombination results in the simultaneous integration of the entire donor plasmid. An alternative system based on cre (cause recombination) recombinase has been shown to be highly effective in mosquitoes, but strongly favors excision over integration. Recombinase-mediated cassette exchange (RMCE) involves the site-specific exchange of DNA flanked by sequences that are substrate for a particular recombinase. In particular, heterospecific lox sites resist in cis recombination (excision). We have developed two *in vivo* quantitative plasmid embryo assays to assess RMCE potential in *Aedes aegypti* using heterospecific pairs of lox sites (locus of crossing-over). *Ae. aegypti* embryos were injected with plasmids designed to quantitatively determine the comparative excision rate of cre upon heterospecific lox sites flanking firefly luciferase ORF. Three candidates exhibited relatively low excision in the presence of cre, these particular sites were then further evaluated for their RMCE potential. All three lox sites tested demonstrated significant RMCE within the embryo inter-plasmid assay. We are currently generating stable transgenic lines that will allow us to determine RMCE potential between a plasmid donor and the different heterospecific lox sites of the multi-functional target cassette. Our end goal is to develop a tool for the transgenic field that will be used to further investigate biological pathways within mosquitoes.

471

INCREASED LIGHT SENSITIVITY UNDER LOW-LIGHT CONDITIONS ASSOCIATED WITH EXTENSIVE AAOP1 REDISTRIBUTION IN *AEDES AEGYPTI*

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We are evaluating the role of vision and circadian cues in the activity and behavior of the disease vector *Aedes aegypti*. At night, a time period for which *Ae. aegypti* is not typically active, low levels of light exposure triggered robust mosquito activity, indicating that light acts outside the realm of circadian input to influence mosquito behavior. The *Ae. aegypti* compound eye is composed of approximately 300 ommatidia, each containing a bundle of eight (R1-R8) photoreceptor cells. The blue light-sensitive Aaop1 is a major rhodopsin of the retina, being expressed in the R1-6 photoreceptors cells. The photosensitive organelle in these cells, known as the rhabdomere, has a microvillar structure that allows a large plasma membrane surface to concentrate rhodopsins and other proteins that function in phototransduction. The Aaop1 rhodopsin is localized to vesicles in the cytoplasm during daylight and absent from the photosensitive membranes of the rhabdomere. At dusk, Aaop1 moves from cytoplasmic multi-vesicular bodies and becomes localized within the rhabdomeres. We examined the influence of circadian rhythms on Aaop1 behavior by exposing the animals to sustained light or precocious dusk. These experiments showed that light itself, not circadian signals, is responsible for the movement of Aaop1. We also carried out protein blot analysis to examine the levels of Aaop1 at different times in the day/night cycle. The results showed that Aaop1 levels gradually increase during the morning period but then declines during the afternoon and evening periods. It is likely that a circadian input influences the change in Aaop1 levels because the decrease was still observed even when light cycles were altered. We also examined the effect of rhodopsin translocation on light sensitivity. There is a correlation between Aaop1 translocation and increased light sensitivity when measured by electroretinogram. The results show there is approximately a 1.5 log unit increase in sensitivity upon translocation of Aaop1 into the rhabdomeres, establishing a correlation between Aaop1 translocation at dusk and increased light sensitivity.

472

THE GENETIC BASIS OF HUMAN HOST CHOICE IN THE MALARIA VECTOR *ANOPHELES GAMBIAE*

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The dominant African malaria vector *Anopheles gambiae* s.s preferentially takes it blood meals from human hosts, often at rates as high as 90% in natural populations. Its adaptation to human hosts has a genetic basis in the olfaction system, which includes several key gene families - the olfacation receptors (ORs), ionotropic receptors (IRs), odorant binding proteins (OBPs). To identify *An. gambiae* genes responsible for human host preference, we conducted a quantitative trait *loci* (QTL) mapping experiment based on backcrosses between the anthropophilic *An. gambiae* and the zoophilic An. quadriannulatus. Backcross females were subjected to a host-choice experiment in an olfactometer in which they were presented with a human and cow odor. Only individuals that selected the same odor on three consecutive days were included in the experiment. A total of ~15,000 individual backcross females were run through host-choice experiments, resulting in two pools totaling 432 mosquitoes with divergent host preferences. We used 38 microsatellite markers to genotype individuals and performed Multiple Interval Mapping using R/QTL. We identified six narrow QTL for human host preference on chromosomes 2 and 3, that together explain 49% of the phenotypic variance. The X chromosome did not contribute significantly to human host preference. A total of 34 ORs, 7 IRs and 21 OBPs are located inside QTL, but three QTL contain only between 4-6 olfaction genes. In addition, a comparison of antennal transcriptomes identified 13 olfaction genes that are located inside QTL and that were significantly higher expressed in *An. gambiae* vs *An. quadriannulatus.*

473

MALE ACCESSORY GLAND FUNCTION IN THE DENGUE VECTOR, *AEDES AEGYPTI*

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Seminal fluid proteins (SFPs) are produced in the reproductive tract of male insects and are transferred, along with sperm, to females during mating. SFPs induce physiological and behavioral changes in the mated female. In the dengue vector mosquito, *Aedes aegypti*, seminal fluid stimulates egg production , induces mating refractoriness, and increases survival rate when injected into females. To date, we have identified 93 male-derived proteins. To learn more about how the male AG functions and how SFPs are produced and secreted, we are studying a novel SFP, AAEL010824. We show that 10824 protein production is specific to the male accessory gland tissue. In addition, 10824 is fully depleted in males after 5 succesive matings , but is almost completely replenished after 48h. To further define 10824's expression pattern, we used 10824 promoter sequences to drive expression of a reporter construct (GFP). Expression is found exclusively in the anterior cells of male accesory glands, suggesting that the gland is composed of more than one cell-type. These results will aid future work to characterize SFP production and secretion in *Ae. aegypti*.

474

TALEN-BASED GENE DISRUPTION IN THE DENGUE VECTOR *AEDES AEGYPTI*

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Aedes aegypti is the primary vector for dengue viruses (serotypes1-4) and chikungunya virus. The inability to control this vector, and thus the disease agents it transmits, has prompted the development of novel genetic-based control strategies. TALE nucleases (TALENs) have been used with great success in a number of organisms to generate site-specific DNA lesions. We evaluated the ability of a TALEN pair to target the *Ae. aegypti* kmo gene, whose protein product is essential in the production of eye pigmentation and confirmed that TALEN-based gene disruption can be a highly efficient process in *Ae. aegypti*, with editing rates between 20-40% which is greater than both traditional transposon-based transformation and phiC31-based recombination. Mutant alleles were associated with lesions of 1-7 bp specifically at the selected target site. White-eyed individuals could also be recovered following a blind intercross of G1 progeny, yielding several new white-eyed strains in the genetic background of the sequenced Liverpool strain. We compared the fitness of these new white-eyed strains in terms of the time of bloodfeeding, larval viability, fertility as a function of egg hatchability and adult mosquito survivorship. We have also investigated the rate of homologous recombination stimulated by TALEN activity by introduction of a green fluorescent protein (GFP) reporter gene into the *Ae. aegypti* germline. We conclude that TALENs are highly active in the early embryos and germline of *Ae. aegypti* mosquitoes, and have the potential to transform how reverse genetic experiments are performed in this important disease vector.

475

BIOCHEMICAL AND MOLECULAR STUDIES OF ALANINE AMINOTRANSFERASE IN *AEDES AEGYPTI* MOSQUITOES

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Alanine aminotransferase (ALAT, EC 2.6.1.2) participates actively in maintaining the alanine-proline cycle between flight muscles and fat body when proline is utilized as an energy substrate during flight in blood-fed *Aedes aegypti* mosquitoes. Additionally, ALAT participates in the ammonia metabolism when *A. aegypti* females are fed a blood or ammonia meal. In order to better understand the mechanisms underlying the ALAT activity in mosquito metabolism, we are currently using multiple approaches. First, we investigated how *A. aegypti* females respond to blood meals supplemented with 0, 2.5, 5 and 10 mM of L-cycloserine, a well-known inhibitor of ALAT in animals. In some experiments, females were starved and then fed a blood meal containing L-cycloserine in the presence or absence of glucose. Survival and motor activity were recorded during a time course (1, 2, 4, 6, 12, 24, 48 and 72 h after feeding). L-cycloserine at 10 mM resulted in high mortality relative to control, with an acute effect during the first 6 h after treatment. A significant reduction of motor activity and blood meal digestion, as well as an increase in futile wing vibration during the first 24 h was observed at all inhibitor concentrations. The starvation and supplementation of glucose in the diet amplified the effect of L-cycloserine. Next, we analyzed the expression pattern of two genes encoding ALAT (1 and 2) in sugar or blood-fed *A. aegypti* tissues. The data show a distinct expression pattern for each gene in mosquito tissues collected at different times after feeding (0-120 h). Finally, we injected dsRNA against ALAT 1 or ALAT 2 into newly-emerged females. In agreement with the results described above, the silencing of either gene by RNAi causes a significant retention of the blood meal, a significant reduction of the nitrogen waste excreted, and an unexpected and dramatic accumulation of nitrogen waste in the midgut. Our studies indicate that *A. aegypti* ALAT plays a critical role in blood-fed *A. aegypti* nitrogen metabolism.

476

YERSINIA MURINE TOXIN IS NOT REQUIRED FOR EARLY-PHASE TRANSMISSION OF *YERSINIA PESTIS* BY *OROPSYLLA MONTANA (SIPHONAPTERA: CERATOPHYLLIDAE)*

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Plague, caused by *Yersinia pestis*, is characterized by quiescent periods punctuated by rapidly spreading epizootics. The classical "blocked flea" paradigm, by which a blockage forms in the flea's proventriculus on average 1-2 weeks post infection, forces starving fleas to take multiple bloodmeals, thus increasing opportunities for transmission and is undoubtedly significant during inter-epizootic periods. Recently the importance of early-phase transmission (EPT) during epizootics has been emphasized. EPT occurs prior to blockage formation and fleas are immediately infectious. While the physiological and molecular mechanisms of transmission are well characterized for the blocked flea model, the pathogen-vector interactions have not been defined for EPT. Within the blocked flea model, two genes, *Yersinia* murine toxin (*ymt*) and the hemin storage locus (*hms*), have been shown to be important for facilitating colonization of the midgut and proventricular blockage within the flea. One proposed mechanism of EPT is the regurgitation of infectious material from the flea's midgut during feeding. Such a mechanism would require bacteria to colonize the midgut, a process that is mediated by *ymt*. *Oropsylla montana*, an important bridging vector in North America, was used in our study to test this hypothesis. Fleas were infected with a mutant strain of *Y. pestis* containing a nonfunctional *ymt* incapable of colonizing the midgut; infected fleas were allowed to feed on SKH-1 mice 3 d post infection. Our results show that colonization of the flea midgut by *Y. pestis* is not required for EPT. Furthermore, our results, in combination with a previous study showing that *hms* is not required for EPT, clearly demonstrate that the vector-pathogen interactions that define EPT are distinct from those in the blocked flea model.

477

HOST CUTANEOUS RESPONSES TO THE ROCKY MOUNTAIN SPOTTED FEVER VECTOR, *DERMACENTOR ANDERSONI,* FEEDING

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Tick salivary glands produce complex cocktails of bioactive molecules that facilitate blood feeding and pathogen transmission by modulating host hemostasis, pain/itch responses, wound healing and both innate and adaptive immunity. In this study, cutaneous responses at Dermacentor andersoni bite-sites were analyzed using Affymetrix mouse genome arrays and histopathology at 12, 48, 96 and 120 hours post infestation (hpi). The microarray data suggests: (1) chemotaxis of neutrophils, monocytes, and other cell types; (2) production and scavenging of reactive oxygen species; (3) keratin based wound healing responses. The histological analysis supported the microarray data findings. At 12hpi, a mild inflammatory infiltrate was present in the dermis, especially concentrated at the junction between dermal connective tissue and underlying adipose tissue. A small lesion was located immediately under the hypostome and likely represents the feeding "pool." Surprisingly, at 48hpi, the number of inflammatory cells had not increased from 12hpi, perhaps mirroring the reduction in gene expression seen at this time point. The feeding lesion is very well defined, and extravasated erythrocytes are readily evident around the hypostome. By 96hpi, the inflammatory infiltrate has increased

dramatically and the feeding lesion appears to have moved deeper into the dermis. At 120hpi, most of the changes at 96hpi are intensified. The infiltrate is very dense, the epidermis is markedly thickened, the feeding lesion is poorly defined and the dermal tissue near the hypostome appears to be loosing its normal architecture. In conclusion, during *Dermacentor andersoni* feeding infiltration of inflammatory cells increases across time concurrent with significant changes in the epidermal and dermal compartments near the feeding tick. The importance of changes in the epidermal layer in the host response to ticks is not known, however, it is possible the host attempts to "slough off" the tick by greatly increasing epithelial cell replication.

478

HUMAN BEHAVIORAL AND ECOLOGICAL RISK FACTORS FOR LYME DISEASE INFECTION ON BLOCK ISLAND, RHODE ISLAND

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Peridomestic exposure to infected *Ixodes scapularis* nymphs is considered the dominant means of infection with tick-borne pathogens in the eastern United States. Previous studies of risk of developing tick-borne infection established a positive association between the density of infected nymphs and Lyme disease cases at the population level. Studies examining the effectiveness of personal protective behaviors have not included measures of tick exposure. This study simultaneously assesses the effect of tick exposure and human behavior in Lyme disease infection risk using a longitudinal serosurvey study on Block Island, Rhode Island. Tick exposure risk at all Island properties was estimated by identifying remotely-sensed landscape proxies that most strongly correlated with tick density at the individual property level. Landscape metrics associated with lawn and shrub edge, density of shrub patches and percent of the landscape occupied by shrubs and the number of patches were found to be most strongly associated with positive serology. Human behavior related risk factors included the average number of hours spent daily outside in tick habitat, and owning a cat that spends time both indoors and outdoors. Age at the time of test was also found to increase risk. Wearing protective clothing during outdoor exposure was protective. A multivariate model including peridomestic shrub patch density (decreased risk), wearing protective clothing (decreased risk), and owning a cat (increased risk) was determined to be the best model based on the lowest Akaike Information Criterion. Our findings emphasize that both environmental risk and human behavior contribute significantly to risk of tick-borne infection. They highlight the importance of accounting for environmental exposure to accurately ascertain the effectiveness of personal protective behaviors. A better understanding of the relative roles of environmental and behavioral risk factors in driving infection with tick-borne pathogens should guide future intervention studies to reduce the risk of these infections.

479

THE INFLUENCE OF LAND COVER, ENVIRONMENTAL AND SOCIO-ECONOMIC FACTORS ON THE SPATIAL DISTRIBUTION OF SCRUB TYPHUS IN TAIWAN

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Environmental factors, including land cover and land use, are known to influence breeding and survival of trombiculid mites and, thus, the spatial heterogeneity of scrub typhus risk, which is transmitted by the mites' larval stage. Here, a spatially autoregressive modelling framework was applied to scrub typhus incidence data from Taiwan, covering the period 2003 to 2011, to provide improved understanding of the spatial pattern of scrub typhus risk and the environmental and socio-economic factors contributing to this pattern. A clear spatial pattern in scrub typhus incidence was observed within Taiwan, and incidence was found to be significantly correlated with several land cover classes, temperature, elevation, NDVI, rainfall, population density, average income and the proportion of the population that work in agriculture. The final multivariate regression model included statistically significant correlations between scrub typhus incidence and average income (negatively correlated), the proportion of land which contained mosaics of cropland and vegetation (positively correlated) and elevation (positively correlated). These results highlight the importance of land cover on scrub typhus incidence: mosaics of cropland and vegetation represent a transitional land cover type which can provide favourable habitats for rodents and, therefore, trombiculid mites. In Taiwan, these transitional land cover areas tend to occur in less populated and mountainous areas, following the establishment and subsequent partial abandonment of agricultural cultivation, due to demographic and socio-economic changes. Future land use policy decision making should ensure that potential public health outcomes, such as modified risk of scrub typhus, are considered.

480

LYME DISEASE RISK IN A SPECIES-POOR ISLAND: LACK OF EVIDENCE FOR THE DILUTION EFFECT

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Biodiversity's role in buffering against human infectious disease is proposed as an ecosystem service applicable to many diseases. The dilution effect hypothesizes that increased host diversity reduces pathogen transmission by reducing direct or indirect contact between hosts. Biodiversity may also influence disease risk if pathogens can specialize to different host species. Lyme disease, caused by the bacterium *Borrelia burgdorferi* and transmitted by *Ixodes scapularis* ticks, is a common zoonotic disease in the northeastern United States. The dilution effect predicts that host communities dominated by *Peromyscus leucopus*, the primary reservoir host, represent the highest risk to humans. Under the host specialization hypothesis, reduced biodiversity may increase disease risk because some strains can persist longer in *P. leucopus* and are more likely to be transmitted to larvae. In a comparative study of a host species-poor community dominated by *P. leucopus* and a host species-rich community, we evaluated support for two specific predictions of these hypotheses: 1) *B. burgdorferi* nymphal infection prevalence and density of infected nymphs is higher in the species-poor than species-rich community, and 2) *B. burgdorferi* genotype diversity is higher in the species-rich than the species-poor community. We collected mammal-derived larvae and questing nymphs in 2010 and 2011 to measure infection prevalence and estimate genotype diversity and richness. We found no differences in nymphal infection prevalence and density of infected nymphs between the two communities, providing no evidence for the dilution effect. We also found high levels of genetic variation in the species-poor community, refuting the host specialization hypothesis. Estimates of genotype diversity and richness of disseminating *B. burgdorferi* strains found primarily in *P. leucopus* were not significantly different between species-poor and species-rich communities. We find little evidence for either hypothesis, suggesting a complicated relationship between biodiversity and disease prevalence.

481

SCABIES PREVALENCE IN KONGWA DISTRICT, TANZANIA

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Scabies is one of the most common communicable diseases in the world, with prevalence as high as 20% in some endemic, resource-poor settings. The mite *Scabies sarcoptii* burrows in the skin, causing itching, and these sores can easily become super-infected with bacteria. Scabies responds to treatment with ivermectin, and scabies cases have been reported to decrease in areas receiving mass drug administration (MDA) with ivermectin, but to date this has only been examined in retrospective studies. We are therefore undertaking a four-year prospective study of scabies prevalence in eight villages in Kongwa District, Dodoma Region, Tanzania, where MDA with ivermectin is being given for lymphatic filariasis. Community-wide baseline surveys were done on all ages in October-December of 2012 by clinical examination of extremities by a trained community health worker. A total of 2269 individuals in 8 villages were examined; of these, most were between the ages of 1-9 (1631, 71.9%). The overall scabies prevalence was 4.3% (97/2269), with most scabies cases being detected in individuals <15 years of age (89 cases in 1-9 year olds, 6 cases in 10-15 year olds). Only 2 cases were seen in individuals >15 years old. Forty-six cases of infected scabies were observed, and skin swabs of scabies sores from all clinically-positive individuals are currently being tested for the presence of bacterial superinfection. Itching was reported in all clinical scabies cases. Scabies prevalence in 7 villages ranged from 2.9 - 5.7%, while one village had a particularly high prevalence of 14.0%. These villages will be followed over the next three years of ivermectin treatment to determine if scabies prevalence drops as a collateral benefit of the lymphatic filariasis MDA program.

482

EPIDEMIOLOGICAL AND GEOGRAPHIC DISTRIBUTION OF TICK-BORNE RELAPSING FEVER BORRELIOSIS IN WEST AND NORTH AFRICA

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Tick-borne relapsing fever (TBRF) is often confused with malaria in Africa and the epidemiology and geographic distribution of the disease are poorly known. Our aim was to investigate the distribution of the vectors and reservoirs of *Borrelia* infections in West and North Africa. From 2002 to 2012, we conducted field surveys in 17 African countries and in Spain. We investigated the occurrence of *Ornithodoros* ticks in rodent burrows in 282 study sites. We collected 1,629 small mammals that may act as reservoir for *Borrelia* infections. Using molecular methods we studied genetic diversity among *Ornithodoros* ticks and *Borrelia* infections in ticks, small mammals and patients with relapsing fever. Of 9,870 burrows investigated, 1,196 (12.1%) were inhabited by *Ornithodoros* ticks morphologically attributable to *O. sonrai, O. erraticus* or *O. normandi*. We collected these ticks in West Africa then North (including Western Sahara) and Spain. In West Africa, the southern and eastern limits of the vector and *Borrelia* infections in ticks and small mammals were 13°N and 01°E, respectively. Molecular studies revealed the occurrence of nine different *Ornithodoros* species, with six of them harboring *Borrelia* infections. The distribution of *O. erraticus* stricto sensu appeared restricted to wet coastal areas of eastern Algeria and western Tunisia and its role as TBRF potential vector was not confirmed. Only *B. crocidurae* was found in West Africa and three - 3 - *Borrelia* species were identified in North Africa: *B. crocidurae, B. hispanica*, and *B. merionesi*. *Borrelia* spirochaetes responsible for TBRF in humans are highly prevalent both in *Ornithodoros* ticks and small mammals in North Africa and northwestern West Africa but *Ornithodoros* ticks seems absent from other regions of West/Central Africa and small mammals are not infected in these regions. Genetic diversity among TBRF *Ornithodoros* vectors is much higher than previously known. Unknown vector / reservoir systems of *Borrelia* infections may exist in wet savanna areas of West Africa.

483

IMMUNE RESPONSES TO CERCARIAL ANTIGENS IN HUMAN POPULATIONS ENDEMICALLY EXPOSED TO SCHISTOSOME INFECTION

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Schistosomes are parasitic helminths which account for over 200,000 deaths per year in sub-Saharan Africa alone. People inhabiting schistosome-endemic regions are repeatedly exposed to infective schistosome larvae (cercariae), which actively penetrate the skin facilitated by the release of excretory/secretory (E/S) molecules. However, the immune response to cercariae and their E/S antigens remains poorly understood in humans. To address this gap in our understanding of schistosome immunobiology two studies were conducted in schistosome-endemic communities. In order to characterise cercariae-specific cytokine profiles, venous blood was collected from a *Schistosoma haematobium*-exposed community in Northern Zimbabwe (n=72) and then cultured with antigens from whole cercariae (cercarial antigen preparation, CAP) for 48 hours. Subsequently 13 CAP-specific cytokines were quantified by enzyme-linked immunosorbent assay (ELISA). This study identified CAP as a potent inducer of pro-inflammatory cytokines (IFN-γ, TNF-α, Interleukin (IL-) 6, IL-8, IL-12p70 and IL-23p19) As well as regulatory IL-10. Importantly these cytokines were further elevated 6 weeks after treatment with the anti-helminthic drug praziquantel (n=21). A subsequent study focused on immune responses to cercarial E/S antigens, which are released in the skin within the first 3 hours post-infection, in order to investigate whether E/S-specific immune responses could be detected in the peripheral blood of people inhabiting an *S. haematobium* and *S. mansoni* co-endemic region of Senegal. In this community venous blood cytokine responses were assayed by ELISA (n=47) and *in vitro* binding of peripheral blood mononuclear cells (PBMCs, n=41) to cercarial E/S antigens of *S. mansoni* was quantified via flow cytometry. Both IL-10 responses to E/S antigens and binding of CD14+CD16+ monocyte subsets to cercarial E/S antigens were greatest in schistosome-infected individuals relative to un-infected people. Together these studies shed light on the immune response to cercariae, the first stage of the schistosome life-cycle encountered by the human host.

484

DETECTION OF MULTIFUNCTIONAL CYTOKINE-SECRETING B REGULATORY CELLS IN MALIAN CHILDREN WITH OR WITHOUT *SCHISTOSOMA HAEMATOBIUM* INFECTION

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The complex interplay between parasite and host induces an immunomodulatory response that may alter the usual host immunity. While B cells are known for antibody production, a subset of B cells produce cytokines, in particular IL-10, and appear to be antigen-activated with the ability to directly suppress effector T cell responses. These immunoregulatory B cells, termed Bregs, may play an important role in regulating parasitic infections but methods of detection are needed. We have optimized a flow cytometric panel that include monoclonal antibodies against CD19, CD3, CD38, CD27, CD24, CD1d, CD5, IgD, and intracellular cytokine detection of IL-10, IL-6, TNF-α, and IFN-γ. Peripheral blood mononuclear cells (PBMC) collected from age-matched Malian children with (SP; n = 5) or without *S. haematobium* (SN; n=5) infection were primed with anti-IgG/IgM antibody or CpG (ODN 2006) followed by a brief stimulation with PMA/ionomycin in the presence of Brefeldin-A. The phenotype and cytokine response of CpG-activated Bregs in SP and SN were of equal magnitude. The majority of the responding Bregs were single-positive for IL-10 (mean±SE: 37.4±1.8%); however double- and triple-positive subsets were also present [IL-10+IL-6+ (36.4±1.8), IL-10+TNF-α (7.7±1.4), and IL-10+IL6+TNF-α+ (17.9±2.4)]. Very few Bregs were found to express INF-γ. However, significantly higher levels of multifunctional Bregs were observed in PBMC from SP children compared to those observed in SN children in response to CpG. Moreover, preliminary results suggest that CpG in conjunction with schistosoma soluble egg antigen (SEA) elicited a 2 to 4 fold higher proportion of Bregs co-expressing IL-6 and IL-10 in SP children. These studies, using an optimized method for the detection of Bregs demonstrate, for the first time, multifunctionality in this B cell subset and heterogeneity in their phenotype. Moreover, they suggest that Bregs in SP children may have higher responsiveness to CpG and CpG + SEA than in SN subjects. The potential role of Bregs in parasitic immunomodulation requires additional study.

485

IN VITRO, HUMAN EOSINOPHILS DOWN MODULATE PERIPHERAL BLOOD MONONUCLEAR CELLS RESPONSES TO *SCHISTOSOMA MANSONI* ADULT WORM ANTIGENS

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Eosinophils have been regarded as terminally differentiated non-replicating effector cells observed in a number of health disorders including parasitic infections and allergic diseases where they play a beneficial role in the host defense against helminth infections or cause a harmful inflammatory response respectively. There is growing evidence that eosinophils can play an additional immunoregulatory role in both adaptive and innate immunity to parasitic infections. We investigated the effects of human eosinophils on peripheral blood mononuclear cells (PBMC) response to *Schistosoma mansoni* adult worm antigen. we have observed that when PBMCs obtained from *S. mansoni* infected adults were examined for cytokine responses to *S. mansoni* adult worm antigen (SWA), eosinophils exerted a down-modulatory effect on schistosome specific responses. The mechanism of this immune-modulation remains to be elucidated.

486

ASSOCIATION OF MODULATORY CYTOKINE GENE POLYMORPHISMS IN RESISTANCE AND SUSCEPTIBILITY TO POLYPARASITISM INFECTION IN ZIMBABWE

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Single nucleotide polymorphisms within the cytokine genes, TNF-α (-308 G/A), IFN-γ (+874 A/T), TGF-β (T/C codon 10 and G/C codon 25) and IL-10 (-1082 G/A and -819 T/C) associated with moderation or severity of parasitic infections were examined in samples from school children aged between 5 - 16 years. About 72.8% were infected with either malaria and/or different helminths. Genotyping was carried out using the ARMS-PCR method. The frequency of TNF-α (GG) associated with low cytokine production was 76.1%, while 22.2% and 1.6% were predictors of medium and high production of TNF-α, respectively. For IFN-γ (+874 A/T), 70.5% were (AA) associated with high cytokine secretion, with 4.4% (TT) and 25.1% (AT) associated with low cytokine production. Limited analysis on the samples also revealed that at the TGF-β locus (T/C codon 10) 88.5% were TT, which predicts high production of the cytokine, whereas 9.2% were CC. Similar analysis at another locus of TGF-β (G/C codon 25) showed that only 2.3% showed GC predicting high TGF-β production. Equal distribution of IL-10 (-819 G/A) and the rare occurrence of allele associated with low IL-10 (-1082 AA) production would suggest moderate to high IL-10 responses in the population. Finally, the high prevalence of TGF-β genotype (TT) predicting high cytokine production and the existence of IL-10 (high producer) might suggest the dominance of an anti-inflammatory environment when faced with acute P.*falciparum* infection in the population. These observations may also suggest a complex interaction between various cytokine gene polymorphisms and high burden parasitic infections in the area.

487

SCHISTOSOMIASIS DURING PREGNANCY IS ASSOCIATED WITH IMPAIRED VACCINE EFFICACY IN KENYAN INFANTS

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Maternal parasitic infections during pregnancy prime the fetal immune response and induce an immunomodulatory phenotype at birth that may affect subsequent immune responses to childhood vaccines. This study determined whether urinary schistosomiasis in pregnant women influenced antibody levels to *Haemophilus influenzae* type B (Hib), hepatitis B (Hep B), diphtheria toxoid (DT) and tetanus toxoid (TT) following vaccination in their offspring. 480 Kenyan women were tested for urinary schistosomiasis (schisto), malaria, lymphatic filariasis and intestinal helminthes during pregnancy. Plasma samples were collected from their offspring every 6 months from birth to 3 years of age and IgG antibody levels to Hib, Hep B, diphtheria and TT vaccinations were measured by ELISA. Although 64% of the pregnant women were infected with one or more parasites, urinary schistosomiasis (30%) was one of the most prevalent infection with consistently detectable lymphocyte responses to *S. hematobium* soluble worm extract (SWAP) as determined by Th2/Th1 cytokine production in cord blood (sensitized, N=239). Other children were born to schistosomiasis infected mothers, but lack cord

blood recall responses to SWAP (putatively immune tolerant, N=45). Some children were born to mothers without schistosomiasis (unexposed, N=91). Using a linear mixed effects models adjusted for maternal age and parity, children characterized with an immunotolerant phenotype due to schisto-driven fetal priming had a lowest vaccine-induced antibody response to Hep B (P=0.001, 0.001, 0.002 and 0.01), DT (P=0.003 and 0.01), and TT-specific IgG (P=0.01, 0.03 and 0.019) at 6, 12, 18 and 30 months of age compared to unexposed children. Thus, urinary schistosomiasis during pregnancy may impair childhood vaccine efficacy. This data highlights the importance of programs to eradicate parasitic infections in pregnant women.

488

LONGITUDINAL ANALYSIS OF ANTIGEN SPECIFIC RESPONSE IN INDIVIDUALS WITH *SCHISTOSOMA MANSONI* INFECTION IN ENDEMIC AREA OF MINAS GERAIS

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Immunoepidemiologic studies have established a clear relationship between IgE and IgG4 antibodies with age, resistance to infection, susceptibility and prediction of infection. It is believed that IgE and IgG4 response to soluble egg antigen (SEA) can be used as a biomarker for infection by *Schistosoma mansoni*, i.e use serological evaluation as basic tool for faster analysis of infection. The purpose of this investigation was to evaluate whether anti-SEA IgE and IgG4 reactivity can be useful as a biomarker to assist monitoring of infection with *S. mansoni*. Between 2001 and 2009, a longitudinal study was performed in Virgem das Graças, Brazil. Parasitological and blood specimens were collected from 127 individuals. Patient sera was tested by ELISA for anti-SEA IgE and IgG4. The schistosomiasis prevalence and geometric mean egg count in 2001 was 59% and 61.05%, respectively and decreased in the following years reaching 26.8% and 8.78% in 2009. The IgG4 anti-SEA reactivity in infected individuals was significantly higher than of uninfected in investigated periods. Analysis of ROC area showed that the IgG4 anti-SEA antibodies were able to predict infection by *S. mansoni* in all study years. Our results showed that the IgG4anti-SEA reactivity can be used as a biomarker for immune monitoring to predict infection outcomes with *S. mansoni* in endemic areas.

489

PROPORTIONS OF CD4+ BUT NOT CD8+ MEMORY T CELLS ARE ALTERED IN OLDER INDIVIDUALS CHRONICALLY INFECTED WITH *SCHISTOSOMA HAEMATOBIUM*

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Protective immunity against human helminth infection develops slowly. Characterisation of parasite-specific responses has focused on effector responses with little work conducted on memory responses in human or experimental studies. Effective generation and maintenance of memory immune responses are central to the development of protective immunity against re-infection and for successful vaccinations. Here we show for the first time, that human helminth infection is associated with altered proportions of the CD4+ memory T cells, with an associated alteration of TH1 responses. Helminth infection does not affect the CD8+ memory T cell pool. Furthermore, we show for the first time in a helminth infection that the CD4+ memory T cell proportions decline following curative anti-helminthic treatment despite increased CD4+ memory cell replication. Reduced accumulation of the CD4+ memory T cells in schistosome-infected people has implications for vaccination programs and may contribute to the reduced vaccine efficacy already reported in helminth infected populations.

490

MULTIPLEX REAL-TIME PCR DEMONSTRATES FOCAL DISTRIBUTION OF *STRONGYLOIDES STERCORALIS* IN DIFFERENT ENDEMIC COUNTRIES

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A major reason why *Strongyloides stercoralis* is highly neglected, even in studies aiming to examine the epidemiology and control of clinically relevant Soil Transmitted Helminths (STH), is the fact infections are simply missed in commonly used diagnostic procedures such as kato-katz stool slide examination. Specific detection and quantification of *Strongyloides* DNA by multiplex real-time PCR has recently been described as a sensitive and highly specific diagnostic method. In the present study we compare prevalence and intensity of *Strongyloide*s infection in different geographical regions using the a standardized real-time PCR procedure. In total more than 4.000 stool samples from both adults and children were collected in eight tropical countries across three different continents. All samples were collected in order to study the epidemiology of helminths within rural or semi-urban communities. DNA isolation, amplification and detection were performed using identical protocols. Results were obtained for *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale* and *S. stercoralis*, of which only the latter will be presented here. The percentage of *S. stercoralis* specific DNA stool positive individuals ranged from less than 1% in Senegal to 10% in Ghana and more than 40% in Mozambique and Peru. In those settings where extensive and *Strongyloides*-dedicated microscopy (i.e. Baermann and copro-culture procedures) was used, PCR-based findings confirmed the detection of L3-larvae. Our data shows a high variation in prevalence and intensity of *S. stercoralis* infection in different communities, ranging from almost zero to extremely high infection levels. In comparison to microscopy, multiplex real-time PCR was found to be a much more sensitive, more reliable and relatively simple high throughput procedure to monitor *Strongyloides* infections in cross sectional surveys. The use of multiplex real-time PCR for the detection and quantification of *Strongyloides* DNA allows for improved comparison of the epidemiology of this infection between different geographical regions.

491

INSULIN/IGF-1-LIKE AND STEROID-NUCLEAR HORMONE SIGNALING ACT IN PARALLEL TO REGULATE DEVELOPMENTAL ACTIVATION OF THIRD-STAGE LARVAE OF *STRONGYLOIDES STERCORALIS*

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Dauer development by the free-living nematode *Caenorhabditis elegans* is considered a model for development by parasitic nematode larvae during host infection. In *C. elegans*, dauer arrest and recovery are regulated in part by insulin/IGF-1-like signaling (IIS) via a steroid-nuclear hormone receptor (NHR) pathway operating downstream. Evidence for ordering of these pathways includes the fact that mutations in the DAF-12 NHR suppress dauer constitutive (daf-c) mutations in signaling kinases in the IIS pathway. Administering a DAF-12 ligand, delta 7-dafachronic acid (DA), to *C. elegans* also suppresses some daf-c mutations, but only partially in the case of strong mutations in DAF-2. This suggests that to a degree, IIS operates parallel to, not upstream of NHR signaling in *C. elegans*. Dauer signaling pathways are conserved in the parasitic nematode *Strongyloides stercoralis*, and IIS is required for developmental arrest and activation of its infective third-stage larvae (L3i). We previously characterized an insulin-regulated PI3 kinase, *Ss*-AGE-1, and showed that its chemical inhibition prevents activation of *S. stercoralis* L3i under host-like culture conditions. Here we asked whether IIS in *S. stercoralis* also works in parallel to NHR signaling by ascertaining whether DA, which promotes activation of *S. stercoralis* L3i *in vitro*, can rescue inhibition of L3i activation by the PI3K inhibitor LY294002. We cultured L3i at 37C and 5% CO_2 in M9 buffer containing either 400 nM DA, which promotes maximal activation, or 400 nM DA plus 100 µM LY294002, which completely inhibits activation. Frequency of activation, indicated by the percentage of larvae ingesting FITC from the medium, was determined after 24 hours. As expected, 92.8% of DA-treated larvae ingested FITC. Significantly, only 8.6% of larvae exposed to both DA and LY294002 ingested FITC. This indicates that chemical inhibition of *Ss*-AGE-1 is not suppressed by administered DA and supports the hypothesis that IIS operates largely parallel to steroid NHR signaling to promote developmental activation of *S. stercoralis* L3i.

492

THE MOLECULAR CHARACTERIZATION OF RIO PROTEIN KINASE-ENCODING GENES FROM PARASITIC NEMATODE *STRONGYLOIDES STERCORALIS*

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RIO protein kinases, a group of newly discovered atypical protein kinases (including three members: RIO1, RIO2 and RIO3) play important roles in cell-cycle progression, 20S pre-rRNA processing and development of many organisms. In spite of their functional significance, there is still a paucity of information on the function of RIO protein kinases in parasitic nematodes. In the present study, full-length cDNA and gDNA sequences of all three RIO protein-encoding genes, Ss-rio1, Ss-rio2 and Ss-rio3, were identified from the parasitic nematode, *Strongyloides stercoralis*. The full-length cDNA of Ss-rio1 comprises 1820 bp, including a 369 bp 5′ UTR, 17 bp 3′ UTR and a 1434 bp coding sequence (CDS). The full-length cDNA of Ss-rio2 is 1572 bp, encoding 524 amino acids. The full-length cDNA of Ss-rio3 has 1736 bp, including a 179 bp 5′ UTR, 36 bp 3′ UTR and a 1521 bp CDS. Two, one and no introns were found in gDNAs of Ss-rio1, Ss-rio2 and Ss-rio3, respectively. Moreover, the in silico analyses of three genes revealed that the promoters of Ss-rio1 with 3128 bp, Ss-rio2 with 1283 bp and Ss-rio3 with 600 bp, contain the conserved motifs, such as a CAAT box, a GATA box, a GC box, an E-box (CANNTG) and a TATA box. In addition, transcriptomic analysis revealed that Ss-rio1 and Ss-rio3 were transcribed at similar levels in most development stages and females of S. strongyloides whereas Ss-rio2 had higher transcriptional levels in free-living and parasitic females than in post free-living L1 and infective L3. Furthermore, the anatomical expression patterns of these three genes were also investigated in transgenic *S. stercoralis*. Ss-rio1 is expressed in some neurons, pharynx, hypodermis and tail phasmidial neurons while Ss-rio2 is expressed in body wall and intestine, and Ss-rio3 in some of the head neurons and hypodermis. The findings from the present study provide a first basis for elucidating molecular functions of RIO protein-encoding genes in the biological processes of parasitic nematodes.

493

PREVALENCE OF *STRONGYLOIDES STERCORALIS* INFECTION IN A REMOTE INDIGENOUS COMMUNITY AS DETERMINED BY ELISA TESTING FROM DRIED BLOOD SPOT FOR ANTIBODIES TO THE RECOMBINANT ANTIGEN NIE

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Parasitologic diagnosis of infection with the intestinal nematode *Strongyloides stercoralis* is relatively insensitive and logistically challenging. Serologic assays based on detection of antibodies to crude larval antigen offer increased sensitivity, but specificity is hampered by cross-reactive antibodies and persistence after cure. Further, standardization of antigen for assays is problematic. The use of recombinant antigen can potentially overcome these problems, and the NIE antigen from *S.stercoralis* has been used widely with good diagnostic sensitivity and specificity. Detection of antibody eluted from dried blood spots has shown utility in large-scale seroepidemiologic studies, and is appealing in children where venipuncture or stool collection is problematic. We adapted an existing NIE-ELISA protocol for the testing of anti-strongyloides antibody response on dried blood spots collected as part of an ivermectin mass drug administration conducted in an Australian Indigenous community. The NIE ELISA was first validated using representative positive, negative, and equivocal time-matched serum samples previously tested using *S. ratti* antigen ELISA. Optimal assay and storage conditions were determined using positive control blood spots, and samples screened with the adapted NIE-DBS-ELISA. Blood spots were stable for several days at 37°C, or following longer-term storage at 4°C, -20°C or -80°C. The sensitivity of the NIE-DBS-ELISA was determined by ROC analysis to be 82%. Of the 219 blood spots tested, 18% were positive for *S. stercoralis*, a similar prevalence to that documented by standard *S. ratti* serology. In representative samples positive for *S. ratti-specific* antibodies, a significant decline in NIE optical density was observed at 6 and 12 months following ivermectin MDA ($p < 0.0001$). No time-associated differences were seen in negative or equivocal samples. This further confirms the high seroprevalence of *S. stercoralis* in remote Australian Indigenous communities, and suggests that collection of dried blood spots may be useful approach for field-friendly diagnosis of strongyloidiasis.

494

STRONGYLOIDES HYPERINFECTION SYNDROME IN AN HTLV-1 CO-INFECTED HIV PATIENT

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An HIV-1 positive, ART naïve, 35- year old male presented to the HIV clinic in Lambaréné, Gabon, with complaints of hemoptysis, persistent after treatment with broad-spectrum antibiotics. Initially there were no constitutional symptoms, the X-thorax showed no particularities, and sputum microscopy was negative for acid fast bacilli. CD4 counts were remarkably high, with repeated values around 1500 cells/mm^3. The FBC

yielded a mild microcytic anaemia with a haemoglobin of 10.4 g/dL without further abnormalities. After local routine periodic treatment for intestinal parasites with albendazole 400 mg once (' deworming'), the clinical picture deteriorated and the patient developed fulminant diarrhea with weight loss over 10% of his initial body weight, vomiting and at a later stage paralytic ileus. The patient remained afebrile during the course of the illness. Rhabditiform larvae of *Strongyloides stercoralis* were identified in the sputum and numerous filariform larvae were found in the faeces. The patient was treated with ivermectin 200µg/kg body weight for 5 days, after which his clinical status improved dramatically. Subsequently, his CD4 count decreased to 100 cells/mm^3 and he was started on ART. Disseminated strongyloidiasis is a severe condition and is associated with corticosteroid use and HTLV-1 infection. HIV-infection does not predispose to the syndrome. In this case, infection with HTLV-1 might have been the reason for the upregulation of CD4 cells, triggered by the auto-infection process of *S. stercoralis*. This masked the severity of immunosuppression by HIV. Clinicians should consider the diagnosis of HTLV-1 in patients from endemic areas with disseminated Strongyloidiasis, both HIV-infected and -uninfected.

495

HOW FREQUENTLY AND TO WHOM SHOULD MASS CHEMOTHERAPY BE ADMINISTERED TO CONTROL SOIL TRANSMITTED HELMINTHS?

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Large-scale treatment programmes for soil-transmitted helminths (STHs) are now being scaled up in endemic areas to reach children in need. There is a concomitant need to decide how best to utilise resources to achieve the biggest impact in terms of reducing transmission in the long term and for effective modelling to support this. We model four age-groups, <2 years, 2-4 years (pre-school-aged children, pre-SAC), 5-15 years (school-aged children, SAC) and adults. The different rates of acquiring infection and of depositing infective stages for the rest of the population to acquire are estimated by fitting the model to a number of age-stratified reinfection studies from countries in sub-Saharan Africa and SE Asia, and to cross-sectional data on intensity of infection by age from single points in time. We describe how the frequency and targeting of effective treatment strategies depend on the overall intensity of infection, the relative intensities of infection in different age groups and the species of helminth. Importantly, we show how the ideal treatment strategy depends on the aim and timescale of the treatment programme. In areas of low to medium transmission (classified on the basis of current WHO guidelines) long-term (>10 years), repeated biannual or annual treatment of >75% of SAC is likely to have large impacts on mean intensity of round worm infections in children and, under certain conditions, on the rest of the population. However, in areas of high transmission significant reductions across the population require the treatment of adults as well as pre-SAC and SAC children. We outline the design of optimum different strategies, extending from SAC to pre-SAC and then adults, under different epidemiologic conditions, including different combinations of Ascaris, Trichuris and hookworm. We also calculate the breakpoints in transmission in terms of the mean worm loads in different age groupings (pre-SAC, SAC and adults) below which treatment can cease. The results are then used to suggest some guidelines for community based control of STH by chemotherapy.

496

HELMINTH INFECTIONS AND MICRONUTRIENTS IN SCHOOLCHILDREN: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Helminth infections and micronutrient deficiencies are both highly prevalent in developing countries. Neither condition typically causes overt disease but they do lead to indirect morbidity and impaired physical and cognitive development. We aimed to systematically review current evidence on the relationship of helminth infections with micronutrient status in schoolchildren worldwide. We included both observational studies and RCTs. We used random effects meta-analysis 1) to estimate cross-sectional associations between helminths and micronutrient status; 2) to estimate anthelminthic treatment effects on micronutrient status, and 3) to estimate effects of micronutrient supplementation on helminth (re)infection. Meta-analyses of observational studies showed a significant association between helminth infections and serum retinol (SMD (standardized mean difference) -0.30 [-0.48;-0.13]) but not serum ferritin (SMD 0.00 [-0.7;0.7]). Conversely, meta-analyses of anthelminthic RCT studies did show a positive effect on ferritin (SMD 0.14 [0.08;0.20]) but not on retinol (SMD 0.04 [-0.06;0.14]). We did not find enough studies to pool data on other micronutrients besides ferritin and retinol. When evaluating helminth (re)infection rates in micronutrient supplementation studies, only multi-micronutrient interventions showed a modest protective effect (OR 0.77 [0.61; 0.97]). In conclusion, we found significant associations between helminth infections and micronutrient status in schoolchildren. Our results showed distinct associations with either serum retinol or serum ferritin. More evidence is needed to further unravel the interrelationship between helminth infections and micronutrient status.

497

TRANSMISSION-BLOCKING INTERVENTIONS ELIMINATE MALARIA FROM LABORATORY POPULATIONS

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Anti-malarial transmission-blocking interventions (TBIs) aim to reduce the prevalence of infection in endemic communities by targeting *Plasmodium* within the insect host. Whilst many studies have previously reported the successful reduction of infection in the mosquito vector, direct experimental evidence that there is an onward reduction in infection prevalence in the vertebrate host is lacking. We report the first experiments using a population, transmission-based study of *P. berghei* in *Anopheles stephensi* to assess the impact of a transmission-blocking drug upon both insect and host populations over multiple transmission cycles. We demonstrate that a selected TBI (atovaquone), which inhibits transmission from vertebrate to insect by only 32%, reduces the basic reproduction number of the parasite by 20%, and in our model system can eliminate *Plasmodium* from mosquito and mouse populations at low transmission intensities. These findings clearly demonstrate that use of TBIs alone can eliminate *Plasmodium* from a vertebrate population, and have significant implications for the future design and implementation of TBIs within the field.

498

TARGETING SEXUAL STAGE MALARIA PARASITES WITH TRANSMISSION-BLOCKING COMPOUNDS IN THE MOSQUITO MIDGUT

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To achieve malaria elimination/eradication, the consensus opinion is that new approaches to drug design are desperately needed. We have undertaken two approaches towards the development of novel malaria transmission-blocking drugs based on the strategy of inhibiting *Plasmodium* development in the mosquito gut either (i) by interfering with obligate cellular interactions between the parasite and the mosquito-midgut epithelium using small molecules that mimic midgut-surface ligands or (ii) by targeting sexual stage parasites with novel compounds that have antimalarial properties. For the first approach, we successfully designed a transmission-blocking small molecule (VS1) that mimics the structure of glycosaminoglycans (GAG), which serve as putative ligands for parasite attachment prior to midgut-cell invasion. For the second approach, we conjugated usinic acid, a dibenzo-furandione acylphloroglucinol derived from lichens with antiparasitic activity, to a number of other compounds including enamines and hydrazones with the goal of improving usinic acid solubility, while enhancing its antimalarial activity and reducing toxicity. Using feeding assays in which mosquitoes were fed with infectious blood, we tested the effect of VS1 and the usinic acid derivatives on *Plasmodium* development in the mosquito and found that the GAG mimetic and multiple usinic acid conjugates dramatically reduced infection intensity in the mosquito. We investigated the molecular mechanism of VS1 using a variety of approaches and found that VS1 binds to the circumsporozoite- and TRAP-related protein (CTRP), a protein expressed by ookinetes essential for midgut invasion. The modes of action of the usinic acid derivatives are currently under investigation, but usinic acid alone has been shown to be a membrane disruptor and to behave as a bifunctional antioxidant-pro-oxidant, depending on its concentration. Both of our approaches have yielded compounds that profoundly inhibit a key step of parasite development, thereby abrogating downstream events necessary for mosquito-to-human transmission.

499

OWNERSHIP AND USE OF INSECTICIDE TREATED NETS AFTER MASS DISTRIBUTIONS TARGETING COVERAGE OF ALL SLEEPING SPACES

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Senegal has made great progress in malaria control through the scale up of malaria prevention and case management interventions and achieved a decrease in all cause child mortality of 40% from 2005 to 2010. In 2010, the Senegal National Malaria Control Program and partners began the implementation of a strategy for universal coverage of long lasting insecticide treated nets (LLINs) with a rolling campaign that covered all 14 regions over three years. Distribution was based on a sleeping space census conducted just before the campaign in each region, with the goal of ensuring one net per sleeping space. Pre-existing nets in good condition counted against the total to be distributed. From May 2010 through March 2013, 6,536,623 LLINs were distributed to 1,480,216 households. A cross sectional cluster sample household survey of 1561 households was conducted in July 2011 in the first six regions (covered from May 2010 through March 2011) to measure campaign coverage, net ownership, and use. The survey found 89% of households received at least one LLIN, with a mean of 4.0 LLINs per household. At the time of the survey, household ownership of at least one LLIN was 94%, and 42% of households owned at least one LLIN per sleeping space. The night before the survey, there were 0.79 LLINs per occupied sleeping space, 82.5% of campaign LLINs were hanging, and 71% of sleeping spaces were protected by an LLIN. Use of LLINs by children under 5 years and pregnant women was 72% and 74%, respectively. Among the general population, 69% in all households and 90% in households with at least one LLIN per sleeping space slept under an LLIN the previous night, demonstrating universal coverage by use when universal coverage by ownership was achieved. While short of the target of 80% use among the general population, Senegal has achieved high LLIN ownership and use. In 2013, a multichannel routine distribution system will be implemented in all regions and the rolling universal coverage campaign will return to the same regions covered in 2010 to maintain high net ownership and use.

500

ASSOCIATION BETWEEN HOUSEHOLD INSECTICIDE-TREATED NET OWNERSHIP AND ALL-CAUSE CHILD MORTALITY IN MALAWI, 2007-2010

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Malawi has made major progress in scaling-up insecticide-treated bed net (ITNs) coverage over the past six years. Demonstrating the protective effectiveness of household ITN ownership for preventing all-cause child mortality (ACCM) under routine program conditions is an important step in the causal pathway towards assessing population-level impact. We used data from the 2010 Demographic and Health Survey (DHS) to assess whether household ownership of an ITN protected against ACCM in Malawi from 2007-2010. We calculated ITN ownership and ACCM retrospectively over this period from the DHS household net rosters, which includes when each net was obtained and/or retreated, and mother's birth histories. Several forms of bias are inherent to this approach. We attempted to mitigate bias due to confounding through exact matching of individuals with and without an ITN based upon residence (urban/rural), mother's education, cluster level vaccination coverage, malaria transmission level, and cluster distance to the nearest health facility. We then evaluated the association between ITN ownership and ACCM in a shared-frailty Cox proportional hazards model while additionally controlling for household wealth, child's age, mother's age, calendar year, parity, cluster level diarrhea prevalence, season (high/low transmission), and monthly rainfall and temperature. The resultant retrospective cohort included 29,492 children <5 who provided 652,775 child-months of observation and among whom there were 821 deaths over the observation period. After controlling for confounders, children in households with an ITN were significantly less likely to die compared to those without one (Hazard ratio (HR): 0.75, 95% confidence interval [CI]: 0.62-0.90). Additionally, ITNs reported as less than 1.5 years old provided greater protection than older nets (HR <1.5 yrs: 0.73, 95% CI: 0.60-0.88, >1.5 yrs: 1.18, 95%CI: 0.70-1.97). These results demonstrate the impact of household ITN ownership on ACCM in this setting and suggest that ITN coverage in Malawi may have contributed significantly to decreased child mortality.

501

RESTORATION OF PYRETHROID SUSCEPTIBILITY AFTER INDOOR RESIDUAL SPRAYING WITH CARBAMATES IN SENEGAL

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Vector control has played a primary role in the dramatic decreases in malaria morbidity in African countries that have scaled up malaria control in the last decade. Mass distribution of pyrethroid-impregnated long-lasting nets (LLINs) has resulted in rapid increases in ownership and use. Indoor residual spraying (IRS), usually with pyrethroids, has been implemented sub-nationally in some countries. However, gains in malaria control are threatened by increasing pyrethroid resistance. Pyrethroids remain the only class of insecticide for LLINs, but many IRS programs have switched to carbamates and organophophates to manage insecticide resistance (IR). Senegal began implementing IRS with pyrethroids in three districts in 2007 and added three more districts in 2010. Monitoring of IRS is done annually in all IRS districts and in select non-IRS sites. By 2010, pyrethroid susceptibility among female *Anopheles gambiae* had fallen in all IRS districts, with mean susceptibility to deltamethrin, lambdacyhalothrin, and permethrin of 54%, 53%, and 34%, respectively compared to 80%, 66%, and 55% respectively, in non-IRS districts. In 2011, a carbamate was sprayed in all IRS districts except one. Post-spray IR testing demonstrated an increase in pyrethroid susceptibility in the carbamate-sprayed districts, with susceptibility to deltamethrin, lambdacyhalothrin, and permethrin of 95%, 88%, and 86% respectively. The district that continued to spray pyrethroid had susceptibilities in 2011 of 72%, 41%, and 35%, respectively, and non-IRS districts had mean susceptibilities of 81%, 73%, and 64%. While susceptibility to pyrethroids increased slightly in non-IRS districts from 2010 to 2011, it increased dramatically in districts sprayed with carbamate. Testing for kdr resistance markers and species identification is ongoing to determine if a change occurred in kdr prevalence or was due to a species replacement. If spraying with a carbamate causes an increase in pyrethroid susceptibility, this may be a strategy to restore vector susceptibility to pyrethroids, prolonging the usefulness of LLINs.

502

MALARIA CHEMOPREVENTION IN HIV EXPOSED INFANTS: A RANDOMIZED CONTROLLED TRIAL OF MONTHLY DIHYDROARTEMISININ-PIPERAQUINE VERSUS MONTHLY SULFADOXINE-PYRIMETHAMINE VERSUS DAILY TRIMETHOPRIM-SULFAMETHOXAZOLE VERSUS NO THERAPY FOR THE PREVENTION OF MALARIA IN A HIGH TRANSMISSION SETTING

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Insecticide treated bednets (ITNs) are currently the only widely adopted intervention for the prevention of malaria in African children. However, the burden of malaria may remain high even in the setting of ITNs in some parts of Africa with high transmission intensity. HIV-exposed children (HIV uninfected children born to HIV infected mothers) are a growing population with the added advantage of trimethoprim-sulfamethoxazole (TS) prophylaxis which has been shown to reduce the incidence of malaria. However, after HIV-exposed children stop breastfeeding and are confirmed to remain HIV uninfected, TS prophylaxis is generally stopped. A cohort of 200 HIV exposed children aged 4-5 months were enrolled using convenience sampling in Tororo, Uganda, a rural area with perennial high transmission intensity. Children received an ITN and daily TS prophylaxis per Ugandan guidelines at enrollment and were followed for all their health care needs 7 d/wk. Approximately 6 weeks after cessation of breastfeeding, 186 children (median age 10 months) who remained HIV uninfected were randomized using an open label study design to one of four treatment arms; no therapy, monthly sulfadoxine-pyrimethamine (SP), daily TS, or monthly dihydroartemisinin-piperaquine (DP). Study drugs were self-administered at home and continued until children reached 24 months of age. The primary end point was the incidence of malaria using passive surveillance. Malaria incidence was compared using a negative binomial regression model with measures of association expressed as the protective efficacy (PE=1-incidence rate ratio) after controlling for age at randomization and the incidence of malaria prior to randomization. Prior to randomization the incidence of malaria was 1.77 episodes per person year at risk (PYAR). After randomization, the incidence of malaria increased to 6.28 episodes per PYAR among those assigned to no therapy. Monthly SP was associated with a PE of 9% (95% CI -35 to 38%, p=0.65), daily TS was associated with a PE of 49% (95% CI 23 to 66%, p=0.001), and monthly DP was associated with a PE of 69% (95% CI 53 to 80%, p<0.001). Following cessation of TS prophylaxis, the incidence of malaria was very high in those children randomized to no therapy despite the use of ITNs. Extending malaria chemoprevention with monthly SP was not effective at preventing malaria, daily TS was associated with modest protective efficacy, and monthly DP was the most effective regimen.

503

EFFICACY AND SAFETY OF IVERMECTIN TO PREVENT MALARIA TRANSMISSION AFTER TREATMENT OF *PLASMODIUM FALCIPARUM* INFECTIONS WITH ARTEMETHER-LUMEFANTRINE: A DOUBLE-BLIND RANDOMIZED CLINICAL TRIAL

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Artemisinin combination therapy (ACT) rapidly clears asexual malaria parasites and developing gametocytes. However, mature gametocytes persist after ACTs and malaria transmission is not prevented. Transmission after ACTs may be reduced by a drug combination that reduces the likelihood that mosquitoes feeding on a human host survive long enough to become infectious. Ivermectin (IVM) may have mosquitocidal properties but has never been used in clinical malaria trials. 120 individuals with asymptomatic *Plasmodium falciparum* mono-infection were randomized to treatment with artemether-lumefantrine (AL) alone or in combination with one or two doses of IVM in a double-blind randomized trial. Clinical safety and lumefantrine plasma levels were determined. On days 1, 3 and 7 after initiation of treatment blood samples were obtained for membrane feeding assays using a median of 94 (interquartile range 92 - 96) *Anopheles gambiae s.s.* and 24 (IQR 23 - 25) *An. funestus* mosquitoes per participant. Mosquito survival during the 10 days post membrane feeding and mosquito infection status were determined. The AL-IVM drug combination was well tolerated. Mosquitoes experienced a 3- to 4- fold reduced survival when feeding 1 day after IVM (p<0.001 for both species) and a 1.4-fold reduced survival when feeding 3 days after IVM (p=0.007). The double dose IVM resulted improved the duration of the mosquitocidal effect and showed a modest reduction in mosquito survival until day 7. In conclusion, our results indicate a significant but short-lived effect of IVM on mosquito survival rates and support a role for IVM in preventing malaria transmission after ACTs.

504

IMPACT OF CHOICE OF ANTIMALARIAL TREATMENT REGIMEN ON THE SELECTION OF DRUG RESISTANCE-MEDIATING POLYMORPHISMS IN *PLASMODIUM FALCIPARUM* IN UGANDA

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Artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) are artemisinin-based combination therapies (ACTs) used to treat *falciparum* malaria. ACTs pair potent, short-acting artemisinins with longer acting drugs that eliminate persisting parasites. However, slowly cleared partner drugs may select for reduced drug sensitivity. Polymorphisms in *Plasmodium falciparum pfcrt* and *pfmdr1* impact upon sensitivity to a number of drugs, with the same mutations that mediate resistance to chloroquine (CQ) and amodiaquine leading to increased sensitivity to other drugs, including lumefantrine and artemisinins. To investigate the selective pressures of AL (the national first-line regimen) and DP on resistance-mediating polymorphisms and to track polymorphism prevalence over time, we studied *P. falciparum* isolated from Ugandan children randomized to treatment with either AL or DP for every episode of uncomplicated *falciparum* malaria from 2008-12. Genotyping utilized a recently optimized ligase detection reaction fluorescent microsphere assay. 950 randomly selected malaria episodes distributed equally over time from each treatment arm were genotyped at 6 *loci* (K76T in *pfcrt*; N86Y, Y184F, S1034C, N1042D, and D1246Y in *pfmdr1)*; studies of additional *loci* (I876V and K1466R in *pfmrp1*) are ongoing. Over time, we found a steady and statistically significant increase in the frequency of the wild-type alleles at *pfcrt*-76, *pfmdr1*-86, and *pfmdr1*-1246 in both treatment arms, indicative of changes in parasite populations in Uganda with decreasing use of CQ and increasing use of AL. However, both the rate of increase and the final proportion of wild-type alleles were much more pronounced in the AL treatment arm in comparison to the DP arm. These results highlight both the changing molecular epidemiology of malaria in Uganda in the context of changing treatment practices and the differential impacts of different therapies on parasite resistance.

505

GENETIC LINEAGES OF EMERGING SULFADOXINE-RESISTANT *PLASMODIUM FALCIPARUM* IN PREGNANCY-ASSOCIATED MALARIA IN MALAWI

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The emergence of drug resistant parasites threatens the efficacy of efforts to prevent pregnancy-associated malaria. In Malawi, *Plasmodium falciparum* parasites bearing the A437G and K540E mutations in the dihydropteroate synthase (dhps) gene ("double mutants") became fixed by the mid-2000s, concomitant with a decline in the efficacy of IPTp-SP. Using parasites from 1997-2010 collected from placental specimens, we monitored for the appearance of parasites bearing the additional A581G mutation in dhps ("triple mutants") and investigated their genetic relationships with double mutant haplotypes using microsatellite markers linked to the dhps gene. Microsatellite profiles were generated at 5 *loci* for 114 *parasitemia*s collected from placental specimens between 1997-2010: 25 wild type parasites (SAKA), 68 double mutants (SGEA), 10 triple mutants (SGEG), and 11 others. Of the major dhps haplotypes, microsatellite heterozygosity (He) was lower for mutant haplotypes SGEA (He 0.454) and SGEG (He 0.485) than wild type SAKA (He 0.798) parasites. By pairwise measures of genetic connectivity, the triple mutant SGEG haplotypes were more closely related to double mutant SGEA parasites by PhiPT (0.036; p = 0.17) and Nei's genetic distance (0.07) than to other haplotypes. Median-joining network analysis of microsatellite haplotypes demonstrated the clustering of triple mutants with haplotypes bearing double mutants. Furthermore, a clonal lineage analysis predicted a shared lineage between triple and double mutant haplotypes that predominate in Malawi. The close genetic relationships between double and triple mutant parasites suggest that the parasites in Malawi that bear the dhps triple mutant SGEG haplotype less likely migrating into Malawi from other sites in East Africa but more likely emerging from established local parasite populations. Further studies are needed to compare triple mutant SGEG haplotypes with other African sites and to characterize the impact of parasites bearing these mutations upon the efficacy of antenatal malaria control strategies.

506

FALCIPARUM MALARIA IN THE GREATER MEKONG SUB-REGION: MAPPING GENE FLOW AND GENOMIC SIGNATURES OF DRUG RESISTANCE

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In response to reports of emerging drug resistance to artemisinins in Western Cambodia, genome-wide association studies have recently been

done to search for the molecular cause(s) of resistance. We now aim to complement these initial efforts by using genomics to study the migration patterns of *Plasmodium falciparum* parasites in and out of the focus of origin of *P. falciparum* artemisinin resistance in Western Cambodia. We used standard population genetics parameters as well as coalescent theory to estimate population structure, divergence, and migration. Coupled with these population and migration analyses, we investigated both the effects of drug resistance-associated positive selection on the genome to look for newly-emerged *loci* possibly selected by exposure to artemisinin. We used cross-population extended haplotype homozygosity to identify regions of the *P. falciparum* genome under positive selection surrounding the pfcrt, pfdhps, pfdhfr, and pfmdr-1 genes responsible for resistance to chloroquine, sulphadoxine, pyrimethamine, and quinolone compounds, respectively. We looked for shared characteristics of signatures surrounding these *loci* and others of unknown origin that could play a role in recently-emerged drug resistance. Using the coalescent migration program Lamarc we observed non-symmetrical migration of parasites from the resistant parasite population in Western Cambodia to points across Southeast Asia. We also found that areas of the genome associated with known drug resistance *loci* have the highest selection scores, and identified multiple additional regions under selection that had high selection scores and no previous association with drug resistance. These results will be discussed in relation to *loci* associated with artemisinin resistance phenotypes in genome-wide association studies.

507

WITHIN-HOST COMPETITION OF MALARIA PARASITES IN HUMANS AND THE FITNESS COST OF DRUG RESISTANCE

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Most infections with the malaria parasite *Plasmodium falciparum* consist of multiple genetically distinct strains, probably resulting from multiple infective mosquito bites in endemic countries. Because these different strains presumably occupy the same ecological niche inside the human body, within-host competition might be expected to occur in mixed-strain infections. If such competition occurs, drug-sensitive strains may suppress transmission of drug-resistant strains, which could slow the evolution of resistance and/or accelerate its decline following the retirement of a failing drug. In order to test the hypothesis that within-host competition occurs in mixed-strain infections, we used quantitative real-time PCR to determine *parasitemia* values of chloroquine-sensitive and chloroquine-resistant *P. falciparum* in 746 blood samples from Ghana (obtained from four locations between 1999 and 2010). Total *parasitemia* did not differ between single and mixed infections (p=0.16), while the density of drug sensitive and resistant parasites was reduced in mixed infections relative to single infections (p=0.0006 and p=0.002 for chloroquine-sensitive and chloroquine-resistant parasites, respectively). These findings suggest that competition does indeed occur when multiple strains infect individual hosts. Further results suggest a fitness cost of drug resistance: first, chloroquine-resistant parasites attained lower densities than chloroquine-sensitive parasites in both single and mixed infections (p=10^-14 and p=10^-5, respectively); second, the frequency of chloroquine resistant parasites in the sampled population dropped from 78% in 2004 (the last year that chloroquine was used in Ghana) to 43% in 2010 following the cessation of drug pressure. This finding corroborates previous observations of decline in resistant alleles following changes in drug policy. In summary: these findings provide evidence that within-host competition occurs in endemic regions and have implications for our understanding of how drug-resistant parasites evolve in different transmission settings.

508

THE EFFECT OF DRUG QUALITY ON THE SPREAD OF ANTIMALARIAL RESISTANCE IN *PLASMODIUM FALCIPARUM*

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Antimalarial resistance has far-reaching global health implications, threatening not only the well-being of patients but the goal of control and ultimately the elimination of malaria from endemic countries. The use of substandard treatments, along with the availability of falsified medicines, undermines this effort. Clinically, substandard treatments are increasingly recognised as an important cause of patients receiving less than the required therapeutic dose of the active ingredient(s); this can arise, either from poorly manufactured products, or falsified medicines. Administration of such subtherapeutic drug concentrations results in selective pressure on the parasite to evolve antimalarial resistance thus enhancing its propagation in a population. The aim of this research was to mathematically model the effects of drug quality on *Plasmodium falciparum* antimalarial resistance in Kenya. For the purpose of this study, poor drug quality was defined to be the use of falsified medicines and substandard treatments. A deterministic mathematical model was developed, with sensitivity analyses and validation methods used to test the model, using data from the Worldwide Antimalarial Resistance Network and the World Health Organisation. The key findings from this model will be discussed, along with the insight the model provides to the current issues of antimalarial resistance control strategies. This study is timely as the effect of drug quality on antimalarial resistance throughout communities is of utmost importance. Antimalarial resistance threatens not only the life of the patient but also the effectiveness of global malaria control programmes and regulatory control of medicines. The relationships identified contribute to the growing body of evidence of the impact that drug quality has on antimalarial resistance and will further inform stakeholders as to where resources can be best utilised.

509

MUTATIONS OF *PFSERCA* GENE AFTER NINE YEARS OF ARTEMISININ COMBINATION THERAPY FOR *PLASMODIUM FALCIPARUM* MALARIA IN THE PERUVIAN AMAZON BASIN

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After the emergence of *Plasmodium falciparum* drug resistance to chloroquine and sulfadoxine-pyrimethamine, Peru was the first South American country to implement artemisinin combination therapy (ACT) based on artesunate (AS) and mefloquine (MQ) in 2001. The *P. falciparum* sarco/endoplasmic reticulum Ca 2+-ATPase (*PfSERCA*) gene was previously reported to be associated with resistance to artemisinin derivatives. The potential emergence of drug resistance requires continual monitoring

for mutations in drug resistance markers; thus, *PfSERCA* was assessed in Peru in 2006-2007 (Bacon et al 2009). In continuation of that study, we analyzed 88 samples collected more recently between 2007 and 2012 in the Peruvian Amazon Basin for 11 mutations in *PfSERCA*: L263D/K, F264L, L402V, E431K, S466N, A623E, A630S, S769N, a G codon deletion in 884, V1168I and a synonymous mutation at C1031(TCC >TGT). We determined that six alleles remained wild type (L263D/K, F264L, E431K, A623E and S769N), and S466N remained very infrequently polymorphic. However, we found substantial increases (>40%) in the frequencies of mutants L402V, V1168I and A630S ($p<0.001$), previously present but below 10%. The synonymous mutation at 1031 also increased from 14% to 30% ($p<0.01$). Finally, the frequency of the G codon deletion at 884 decreased from 77% to 22% ($p<0.001$). Eight genotypes were documented in the samples from 2006-2007, including a wild type (4%); the most frequent were the G codon deletion at 884 (69%), and the synonymous mutation at 1031(11%). In contrast, eleven genotypes were identified without any wild types in the samples from 2007-2012. The two most frequent were the 3M 402/630/1168 (n=39, 44%) and the 2M 630/1030 (n=24, 27%). Sixty percent of the current samples were identical to previously documented strains while 40% represented new genotypes. The observed changes in the *PfSERCA* genotypes suggest the circulation of different *P. falciparum* strains, perhaps in response to ACT. Therefore, the role of selective pressure by AS and MQ on these changes should be evaluated and monitored prospectively.

510

CHALLENGES IN THE INTERPRETATION OF DENGUE VACCINE TRIAL RESULTS

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Several previously published hypotheses have been proposed to explain the unexpected results of the first completed clinical trial of a vaccine against dengue virus. The vaccine was effective in reducing the incidence of clinical disease caused by dengue serotypes 1, 3 and 4, but failed to reduce the incidence of dengue-2 (DENV-2). The authors of the study propose potential explanations including an antigenic mismatch between the parental strain of the DENV-2 component and currently circulating DENV-2 viruses in Thailand, an increased role for immunity to non-structural proteins in DENV-2 that this vaccine does not induce and a lack of correlation of measured neutralizing antibody and protective immunity. However, we believe that in addition to questioning the immune response elicited by the vaccine, it is important to discuss the interpretability of efficacy results for dengue vaccine trials that are based exclusively on clinical outcomes. A previously publisher study measured vaccine efficacy against clinically apparent infection. This is distinct from vaccine efficacy against infection, and potentially a very important distinction. To explore the agreement between vaccine efficacies against infection and against clinical infection --under different assumptions of the impact of prior heterologous immunity on the probability of symptomatic disease-- we developed an analytical probabilistic framework that allows these efficacies to be quantified. Our results suggest that the vaccine efficacy against clinically apparent infection often leads to large underestimates of the vaccine efficacy against infection but can also lead to overestimates depending upon the tradeoff between preventing infections and inducing immunity that can predispose individuals to a more severe outcome. Discarding or moving forward with vaccine candidates purely on the basis of its efficacy against clinical disease could lead to prematurely abandoning vaccines that have promising biological action or moving forward with an overly optimistic estimate of a vaccine's impact.

511

POTENTIAL OPPORTUNITIES AND PERILS OF IMPERFECT DENGUE VACCINES

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The ideal dengue vaccine is one that has high and equal efficacy against all four serotypes. However, results of a recent Phase IIb trial indicate that the vaccine candidate furthest along in development protects against serotypes 1, 3 and 4 but not serotype 2. While partially effective vaccines may have a positive impact on morbidity and mortality, particular profiles could result in increased clinical disease due to antibody dependent enhancement. The potential population-level impacts of a partially effective vaccine have not been explored. We developed a four-serotype, age-specific compartmental dengue transmission model that takes into account cross-protection and interaction between serotypes. We calibrated the model to capture transmission dynamics in traditionally hyperendemic setting (Rayong, Thailand), using data from a recently conducted age-stratified serological survey and age-specific incidence data. We also considered scenarios where the disease has re-emerged more recently (Mexico, and Brazil). We used the model to assess the potential impact of partially effective vaccines at the population level and to estimate direct and indirect vaccine effects. Crucially, we evaluated the effects that heterogeneities in pathogenicity, transmission intensity and enhancement between serotypes may have in the presence of mass vaccination campaigns. In the majority of scenarios explored, the impact of partially effective vaccines was positive and resulted in 50% or greater reductions in the number of cases. This was true even of vaccines that we would not expect to be licensed due to poor or incomplete immune responses. Our results also show that partially effective vaccines can have significant impacts on the mean age of cases and on serotype distributions, due to reduced competition for susceptible individuals. The magnitude of direct vs. indirect vaccine effects depended on the particular scenario explored. Dengue vaccine development efforts have focused on the development of tetravalent vaccines. Our results suggest that despite the virologic and immunologic characteristics of dengue, partially effective vaccines have the potential to be important tools for dengue control. Consideration of imperfect vaccines will require careful characterization of the epidemiology of dengue in each place.

512

THE LIVE ATTENUATED TETRAVALENT DENGUE CANDIDATE VACCINE TV003 IS WELL TOLERATED AND HIGHLY IMMUNOGENIC IN FLAVIVIRUS-EXPERIENCED SUBJECTS

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Dengue virus (DENV) has become the most important arbovirus worldwide with nearly 400 million infections occurring annually. To develop a live attenuated tetravalent (LATV) dengue vaccine with the most favorable safety and immunogenicity profile, the US National Institutes of Health evaluated different monovalent and tetravalent DENV candidate vaccines. Following a single subcutaneous dose of admixture TV003 in flavivirus-naïve subjects, 74% of vaccinees became seropositive to all 4 DENV serotypes and 92% became seropositive to ≥ 3 serotypes. Prior to evaluating TV003 in dengue endemic areas, its safety, replication,

and immunogenicity were evaluated in flavivirus-experienced subjects. Volunteers with a documented history of flavivirus exposure or flavivirus immunization were recruited in the Baltimore MD and Burlington VT areas. 56 subjects were enrolled in this trial; 40 subjects received TV003 and 16 received placebo. Each of the components of TV003 was given at a dose of 1,000 PFU. Six months after receipt of the first dose of vaccine, subjects were challenged with a second dose. They were followed in an identical manner after both doses. Viremia and safety labs were assessed on days 0, 3, 6, 8, 10, 12, 14, and 16 after each immunization. Specimens were collected for serological analysis on study days 0, 28, 56, 90, 150, and 180 post-immunization. Sixty percent of all vaccinees had at least one vaccine virus recovered from the blood following the first immunization; none had detectable vaccine virus in the blood following the second dose. Although the mean peak titer of two of the vaccine components was slightly higher in flavivirus-experienced subjects compared with flavivirus-naïve historical controls, there was no significant difference in the adverse event profile. Following the first immunization, the vaccine induced a tetravalent neutralizing antibody response in 85% of vaccinees and a trivalent or better response in 100% of vaccinees. Complete safety and immunogenicity data following the first immunization will be discussed as will differences noted between the responses in flavivirus-naïve (historical data) and flavivirus-experienced subjects. The safety, absence of viremia, and immunologic response of vaccinees to the challenge dose of vaccine will also be discussed.

513

THE TYPE-SPECIFIC NEUTRALIZING ANTIBODY RESPONSE ELICITED BY A DENGUE VACCINE CANDIDATE IS FOCUSED ON TWO AMINO ACIDS OF THE ENVELOPE PROTEIN

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Dengue viruses are mosquito-borne flaviviruses that circulate in nature as four distinct serotypes (DENV1-4). Severe clinical manifestations of disease are predominantly associated with a secondary infection by a heterotypic DENV serotype. The increased risk of severe disease in DENV-sensitized populations significantly complicates vaccine development, as a vaccine must simultaneously confer protection against all four serotypes. Eliciting a protective tetravalent neutralizing antibody response is a major goal of ongoing vaccine development efforts. However, a recent large clinical trial of a candidate DENV vaccine revealed low protective efficacy despite eliciting a neutralizing antibody response, highlighting the need for a better understanding of the humoral immune response against DENV infection. To identify epitopes recognized by serotype-specific neutralizing antibodies elicited by monovalent DENV1 vaccination, we constructed a panel of over 50 DENV1 structural gene variants containing substitutions at surface accessible residues of the envelope (E) protein to match the corresponding DENV2 sequence. Amino acids involved in recognition by serotype-specific neutralizing antibodies were identified as DENV variants with reduced sensitivity to neutralization by DENV1 immune sera but not by cross-reactive neutralizing antibodies elicited by DENV2 vaccination. We identified two mutations that contribute significantly to type-specific recognition by polyclonal DENV1 immune sera. Longitudinal and cross-sectional analysis of sera from 24 participants of a phase I clinical study revealed a markedly reduced capacity to neutralize a DENV1 variant containing the two mutations. Sera from 77% of subjects recognized the DENV1 variant and DENV2 equivalently (<3-fold difference). These data indicate the type-specific component of the DENV1 neutralizing antibody response to vaccination is strikingly focused on just two regions of the E protein. This study provides an important step towards deconvoluting the functional complexity of DENV serology following vaccination.

514

DENVAX ELICITS TYPE-SPECIFIC AND BROAD NEUTRALIZING ANTIBODY RESPONSES REACTIVE TO DIVERSE DENV ISOLATES

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An important prerequisite for successful vaccination against dengue viruses (DENV) is the induction of neutralizing antibody responses to all four serotypes. Ideally, such a response should be type-specific and have a broad cross reactive potential against genetically and geographically diverse DENV isolates from each serotype. In our studies we characterized; i) the potential of DENVax to elicit type-specific neutralizing antibody responses, and ii) the breadth of neutralizing antibody responses elicited by a tetravalent DENV vaccine based on the DENV-2 PDK-53 cDNA clone genetically engineered to express the prM and E genes of DENV-1, -3, and -4 (DENVax). Depletion of anti-DENV-2 neutralizing antibodies from DENVax immune serum had no significant impact on the neutralizing titers to the other three serotypes. We also used a panel of 24 DENVs collected the last two decades from different tropical and subtropical regions of the world to screen immune serum elicited after DENVax immunization of non-human primates or naïve individuals from a phase I clinical trial. Immune serum from both species collected after secondary immunization exhibited broad neutralizing activity across all the DENV serotypes. Taken together these data suggest that DENVax could elicit type-specific neutralizing antibody responses and be potentially effective in protecting against contemporary DENV strains circulating in endemic areas.

515

A PHASE 2 AGE-DE-ESCALATION CLINICAL TRIAL OF A RECOMBINANT LIVE ATTENUATED TETRAVALENT DENGUE VACCINE (DENVAX) IN HEALTHY VOLUNTEERS FROM ENDEMIC COUNTRIES

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We have been developing a tetravalent, live attenuated dengue vaccine (DENVax) consisting of a molecularly characterized, attenuated DENV-2 strain and three chimeras in which the prM and E genes of the attenuated DENV-2 were substituted with those of DENV- 1, -3 or -4 viruses. We have previously demonstrated that tetravalent formulations of DENVax were safe and immunogenic after either subcutaneous or intradermal administration in Phase 1 clinical trials in healthy, flavivirus negative adults. We have now evaluated DENVax in a Phase 2 age-de-escalation clinical trial conducted in healthy volunteers (ages 1.5-45 years-old) in four endemic countries (Puerto Rico, Colombia, Thailand and Singapore). DENVax was administered at 0 and 3 months by subcutaneous injection. A total of 148 subjects (76M: 72F) were dosed in four sequential age cohorts

with data from each cohort reviewed by an independent Data Safety Monitoring Board before the next younger cohort was dosed. There were no related serious or severe adverse events (AEs), and no discontinuations due to vaccine-related AEs. The most common AEs included headache (18%), upper respiratory infections (15%) and pharyngitis (14%). The most common laboratory changes were decreased hemoglobin (47%, all Grade 1), decreased fibrinogen (17%), decreased WBC (14%, all Grade 1). Transient local, reactogenicity was noted in about 25% of subjects. Thus, the vaccine was well-tolerated with mostly mild and transient local or systemic reactions. Preliminary analysis reveals that DENVax induced significant neutralizing antibody responses to all four dengue viruses after one or two administrations: 98.8% of subjects were seropositive for three or more dengue viruses and 87.2% were seropositive for all four dengue viruses one month after second dose. This study highlights the safety and immunogenicity of the tetravalent DENVax vaccine in children and adults in dengue endemic countries. DENVax warrants further evaluation in Phase 2b/Phase 3 efficacy studies.

516

PRECLINICAL AND CLINICAL TESTING OF A RECOMBINANT SUBUNIT VACCINE FOR DENGUE

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Dengue viruses are a major cause of morbidity and mortality throughout the tropics and subtropics. It is estimated that more than 120 countries currently have endemic dengue virus transmission. Each year there are at least 50-100 million infections, of which 2.1 million are clinically severe, resulting in more than 21,000 deaths. While a licensed dengue vaccine is not yet available, several vaccine candidates designed to protect individuals against dengue virus-induced disease are currently being evaluated in clinical trials, including a tetravalent recombinant subunit vaccine. Preclinical studies of this recombinant subunit vaccine have been conducted in non-human primates to evaluate the immunogenicity and efficacy of tetravalent formulations. Vaccine formulations have been evaluated in both dengue naïve and experienced animals. These preclinical studies have shown the capacity of the recombinant proteins to induce balanced tetravalent responses without evidence of interference. Data from these preclinical non-human primate studies will be presented. The vaccine candidate is also being tested in a Phase 1 clinical trial in healthy, flavivirus-naïve, adults. An update on clinical trial status will also be provided.

517

UPDATE ON EVALUATION OF AN ATTRACTIVE LETHAL OVITRAP (ALOT) AGAINST *AEDES AEGYPTI* FOR DENGUE CONTROL IN IQUITOS, PERU

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We have developed a lethal ovitrap (Attractive Lethal OviTrap = ALOT) for dengue prevention, with concentration on identification of mosquito oviposition attractants and stimulants. Since June 2011, we have tested the ALOT in a field trial in dengue-endemic Iquitos, Peru. The study design was a prospective nonrandomized controlled trial in two cohorts of Iquitos residents from two comparable city neighborhoods each of 2500 houses selected as either intervention or control zones. Traps were placed at a density of ~3 per residence, with ~85% participation in the intervention area. Local ministry of health fumigation to control adult mosquitoes was ongoing in both areas during the study. Entomological indices were monitored in participating households at 3 month intervals, and individuals were monitored serologically, both through a longitudinal survey (at months 0, 12, 24) and through 3X weekly febrile surveillance. One year into the trial, dengue incidence as measured by febrile surveillance was 75% lower (0.26% vs. 0.97%) in the intervention area compared to the control area ($p<0.0001$). Longitudinal surveillance showed that people in the ALOT area were 25% less likely to contract symptomatic or asymptomatic DENV than those in the control area. Incidence was 12.67 vs. 17.05 in ALOT vs. Control areas, respectively ($p=0.03399$). The proportion of nulliparous females (compared to parous and/or gravid females) was significantly higher in the ALOT area, supporting the removal of older egg-laying mosquitoes from the population. This held true for both the targeted species, *Ae. aegypti* ($p<0.0001$), and for non-target *Culex* species ($p=0.0030$). Finally, if the ALOT differentially attracts females vs. males, we would expect a skewing of the sex ratio over time to show fewer females relative to males. There was a highly significant difference in sex ratio ($p<0.0001$), between the ALOT and Control sites. The ratio in the ALOT area started at 50:50 and is now at 60:40 (male to female) while the Control area has not changed from 50:50 since start of the project in June 2011.

518

RIVER BOATS CONTRIBUTE TO THE SPREAD OF *AEDES AEGYPTI* MOSQUITOES IN THE PERUVIAN AMAZON

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In the Americas, as in much of the rest of the world, the dengue vector *Aedes aegypti* is predominantly found in urban areas. Its presence in rural areas is more limited, and the factors favoring its potential geographic expansion to rural and smaller urbanized settings are poorly understood. In the Peruvian Amazon, this vector has been expanding its range into rural communities over the last 5-10 years. Understanding *Ae. aegypti* geographic expansion is important for anticipating the future range expansion of dengue and other viruses transmitted by the mosquito. To examine the hypothesis that accidental transport of mosquitoes by human vehicles plays a significant role in such expansion, we measured adult and immature *Ae. aegypti* abundance in a total 97 fluvial and terrestrial vehicles departing the city of Iquitos. River transit included small public transit boats (peque-peques), medium-sized boats transporting cargo and passengers over short (avg. of 96.2 km) distances (lanchitas) and large boats transporting cargo and passengers over long (avg. of 349.5 km) distances (lanchas). Road vehicles included public transit buses (combis) and group taxis (colectivos). Each vehicle was surveyed for *Ae. aegypti* adults (aspiration) and immatures (pupal surveys) at three different ports and two different bus/ taxi departure points. Our results show that *Ae. aegypti* adults and immatures are most prevalent on lanchas (13/17, 76.5%), lanchitas (5/15, 33.3%), and combis (3/16, 18.8%), while the remaining vehicle types were negative (Fisher's exact test across all vehicle types $p<0.0001$). Our data suggest that large, slow-moving river boats are much more likely than other types of vehicles to contain mosquitoes in general and *Ae. aegypti* mosquitoes specifically. Furthermore, it is evident that there is constant *Ae. aegypti* introductory pressure from Iquitos to riparian rural communities. Additional studies are underway to determine the role of local ecological conditions in the range expansion of the *Ae. aegypti*.

519

REEMERGENCE OF *ANOPHELES FUNESTUS* AS A VECTOR OF *PLASMODIUM FALCIPARUM* IN WESTERN KENYA

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Malaria causes significant morbidity and mortality in western Kenya despite public health efforts to reduce its prevalence. Historically, the primary malaria vectors in the region have been *Anopheles funestus*, *An. gambiae s.s.* and *An. arabiensis*. Of these species, *An. funestus* populations declined the most after the introduction of insecticide treated bed nets (ITNs) in the 1990s in Asembo, and collections of *An. funestus* in the region remained low until at least 2005. We investigated indoor resting densities, human biting rates, and malaria infection rates of *An. funestus* and other malaria vectors in Asembo to determine if *An. funestus* populations and malaria transmission rates remained low or had increased despite intensification of ITN distribution. Additionally, we measured the sensitivity of the vector populations to pyrethroid insecticides in LLINs through standardized bioassays. We sampled *Anopheles* mosquitoes using the pyrethrum spray catch method (PSC) in 2010 and 2011 and the human landing catch method in 2011 and identified all specimens to species using both morphological and molecular methods. We tested female *Anopheles* for *Plasmodium falciparum* sporozoites by ELISA and identified blood meal hosts by direct sequencing of the vertebrate mitochondrial cytochrome B gene. We performed insecticide susceptibility assays on wild caught *Anopheles* adults collected from Asembo by exposure to permethrin, deltamethrin and bendiocarb. Contrary to findings during the early years of ITN use in Asembo, the majority of the *Anopheles* collected in our study were *An. funestus*. Female *An. funestus* had characteristically high *P. falciparum* sporozoite rates and showed nearly 100% anthropophily. Female *An. funestus* were found more often indoors during HLC sampling and had relatively low mortality rates during insecticide bioassays. Together, these results are of serious concern for public health in the region, indicating that *An. funestus* may once again be contributing significantly to the transmission of malaria in this region despite the relatively widespread use of ITNs.

520

DETERMINING THE OPTIMAL MIX OF MALARIA CONTROL INTERVENTIONS IN THE HIGHLANDS OF WESTERN KENYA THROUGH SIMULATION MODELS

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Tools to assist malaria control professionals in their decision making processes will become increasingly important as calls continue for better targeting for malaria control interventions. A stochastic simulation modelling platform, OpenMalaria, was used to simulate the impact of combinations of a range of existing and potential future malaria control interventions implemented in the highlands of western Kenya. Combinations of interventions to include in simulations, as well as their coverage levels and deployment schedules, were chosen in collaboration with malaria control personnel in the study area to correspond to a 2011-2012 intervention evaluation trial. The model and baseline scenario were parameterized based on a previously-published, validated model of malaria epidemiology and control in the study area. Simulations ran for a period of five years on a population of 1,000 individuals using an ensemble of 14 model variants to address model uncertainty. The impact of intervention combinations was evaluated by estimating, after five years of implementation, the simulated annual average averted cases of uncomplicated malaria in children under five, reduction in all-age average parasite prevalence, and reduction in annual average EIR compared to the corresponding simulated outputs of the baseline scenario. The combination of interventions resulting in the largest simulated reduction in all indicators was long lasting insecticide treated net (LLIN) use by 80% of the population, 90% of households covered by indoor residual spraying (IRS) with deployment starting in April, and a mass screen and treat program covering 80% of schoolchildren implemented twice per school term. Despite moderate observed use in the population, simulations show LLINs and not IRS account for the majority of impact on transmission. While deployment of IRS starting in April ahead of the rainy season resulted in the largest simulated reduction in annual average EIR, starting IRS in May showed a greater impact on averting uncomplicated cases in children under five and reducing all-age parasite prevalence. These results have the potential to assist malaria control program managers in the study area if they wish to add new or change the implementation of current interventions.

521

HOUSE ENTRANCE AND EXIT MOVEMENT PATTERNS, HOST PREFERENCE, AND WING GEOMETRY OF TWO FIELD POPULATIONS OF *ANOPHELES DARLINGI* ROOT THAT REPRESENT TWO GENOTYPES

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Anopheles darlingi Root, a major vector for malaria in Central and South America, has been shown to have two distinct genotypes: a northern lineage (Belize, Guatemala, Colombia, Venezuela and Panama) and a southern lineage (Amazonia and southern Brazil). To test whether behavioral differences in house entrance and exit movement patterns and host preference could be observed between each genotype, two field populations of *An. darlingi* that represented each genotype were observed. In Cayo District, Belize (representing the northern lineage), peak house entry occurred between 7:00-8:00 p.m. and 5:00-6:00 a.m. and peak exit occurred between 7:00-8:00 p.m. In Loreto Department, Peru (representing the southern lineage), peak house entry occurred between 10:00-11:00 p.m. and peak exit occurred between 11:00-12:00 a.m. Entrance and exit behavioral patterns were significantly different between the two populations of *An. darlingi* [log-rank (Mantel-Cox) $P < .001$]. The Belize population of *An. darlingi* was observed to have a significantly higher number of mosquitoes collected from a house with a human host than from a house with a pig host ($P < .025$). In Peru, there was no significant difference in the number of *An. darlingi* collected from a house with a human host and a house with a pig host. *Anopheles darlingi* collected from each experiment were analyzed using geometric morphometrics to compare wing shape and showed statistically significant shape variation between each geographic population ($P < .001$). A subset of *An. darlingi* samples from each site was sequenced to verify the genotype of each population. Information from these studies can be used to assess the relationship between genotype and host-seeking behavior and can be used for regional vector risk assessment.

522

A NEW LONG LASTING INSECTICIDAL NET (LLIN) THAT COMBATS PYRETHROID RESISTANCE IN MOSQUITOES

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The large control programmes around the world and especially in Africa to combat malaria and reduce deaths predominately in children of >5

years old have employed Long Lasting Mosquito Nets (LLINs) which contain pyrethroid insecticides and are wash-proof for >20 washes. These have been largely responsible for reducing malaria deaths from c2 million/ year to <670,000. However pyrethroid resistance is increasing especially in parts of Africa and indications are that it is reducing the efficacy of this intervention. LLINs only use pyrethroids since low toxicity is essential due to the proximity of the people and especially children sleeping under them, therefore alternatives are limited. It has been known for many years that the synergist piperonyl butoxide (PBO) has a good impact on the metabolic based resistance mechanism in mosquitoes and is routinely used in household aerosols and space sprays, however it had not been used on nets due to the difficulties with stability and obtaining parallel degradation and loss curves with the pyrethroid over time. This new product Olyset Plus® has after much research and development achieved this and it contains both permethrin and PBO within the polyethylene fibres on all 5 surfaces of the rectangular net.The mesh size is smaller so it can be used against sandflies and the surface regeneration time of the actives has been speeded up the so there is minimal time after washing before it becomes active again. Trials have been conducted in both the laboratory and field against susceptible and multi-resistant mosquitoes. In comparison to a similar net without PBO the Olyset Plus performed much better even against susceptible mosquitoes due to faster penetration of permethrin through the cuticle aided by the PBO acting as a solvent and the suppression of P450 enzymes present naturally in susceptible mosquitoes. Independent trials conducted in both Benin and Cameroon showed excellent impact against both metabolic resistant population of mosquitoes and in Benin ones showing (Knockdown resistance) Kdr which was surprising.

523

LLINS AND IRS ALONE WILL NOT ELIMINATE MALARIA IN THE SOLOMON ISLANDS: THE EVIDENCE TO SUPPORT NOVEL INTERVENTION USE

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The Solomon Islands are undertaking intensified malaria control and localised elimination. The key interventions are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), which work by killing mosquitoes after they enter houses. We therefore studied the biting behaviour of the primary malaria vector: An. farauti. This research was conducted in Central Province during 2011-2012. *Anopheles farauti* were observed to bite humans outside of houses (65% of the time) and early in the night (with 82% of biting before 9PM). After adjusting for when humans were inside houses, the percentage of exposure to mosquito bites which occurred indoors was only 16%. Mark-release-recapture experiments were then conducted to determine if the phenotypes for early and outdoor feeding are fixed in individual mosquitoes. Analyses showed that the phenotypes for time and place of feeding in consecutive feeding cycles were not significantly different from that observed for the entire population, consistent with a hypothesis that there is no structuring of the population. Hence, all individual An. farauti exhibit an equal probability to enter houses, and thus be exposed to the insecticides in LLINs and IRS during each feeding cycle. However, as *An. farauti* mainly bite outdoors and early in the night the efficacy of vector control tools deployed inside houses, such as LLINs and IRS, is significantly reduced. As such, there is an urgent need for complementary vector control tools that can target mosquitoes that feed outdoors and early in the night.

524

SPATIO-TEMPORAL ANALYSIS OF LEPTOSPIROSIS INCIDENCE IN A HIGH-RISK URBAN SLUM COMMUNITY IN BRAZIL

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Leptospirosis has emerged as an important health problem in slum settlements worldwide. Yet, the lack of prospective information on transmission sources in these settings has hampered the development of effective interventions. A cohort of 2,003 residents from an urban slum community in Salvador, Brazil was recruited in 2004 and followed for a four-year period. Household interviews and surveys were performed annually to evaluate risk behaviors and geocodify place of residence and potential transmission sources. Annual serosurveys were performed to identify incident leptospiral infections. We fit a multivariate model that included random effects accounting for unexplained inter-person, spatial and temporal variation, whose structure can help to identify anomalous areas of high or low risk that can be further investigated. A total of 1094 individuals were followed for 4 years; 1724 completed at least one year of follow-up. The annual infection rate was 43.24 per 1,000 persons (95% CI 38.04-48.97), in a total of 5573 follow-up years. We identified household elevation - an inverse proxy for flood risk (OR 0.98 per meter; 95% CI 0.96-0.99), and reported sighting of rats near the household (1.57; 1.09-2.27), as significant independent risk factors in a model which included also age, male gender and illiteracy as significant covariates. Furthermore, reported contact with floodwater was associated with lower infection risk (0.49; 0.28--0.84), while contact with both floodwater and mud was independently associated with a higher risk of infection (2.27; 1.13-4.71). Although there was substantial temporal variation in infection risk (range 1.92-11.04 per 1000 persons), regions of highest risk did not vary year to year during the study period, and the analysis identified discrete locations with consistently higher or lower risk than was explained by the model. In slum communities, deficiencies in the peridomiciliary infrastructure, such as open sewers and poor drainage systems serve as persistent sources for transmission of leptospirosis. Furthermore, efficient transmission appears to require the specific interaction between flooding and exposed soil, the environmental reservoir for leptospires. Together these findings suggest that there are defined "hot spots" for transmission within slum communities and that targeting these sites by isolating soil from flood run off may be an effective prevention approach.

525

THE USE OF MOBILE HEALTH (MHEALTH) TECHNOLOGY IN AN ORAL CHOLERA VACCINATION CAMPAIGN IN RURAL HAITI

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In mass vaccination campaigns, large volumes of data must be managed efficiently and accurately. In an oral cholera vaccination (OCV) campaign in rural Haiti during an ongoing epidemic, we used a mobile health (mHealth) system to manage data on 50,000 participants in 2 isolated communities. Data were collected using Samsung Galaxy 7.0 Plus tablets by 50 enumerators and 20 supervisors. Teams pre-registered and distributed

vaccine cards to eligible residents in 9,517 households during a 13-day census in February 2012. Using the tablets' barcode-scanning function, we tracked participants by unique numeric barcodes on their vaccine cards. First stored on devices, data were then uploaded nightly via Wi-fi to a web-hosted database. During the campaign from April to June 2012, residents presented their cards at vaccination posts and their barcodes were scanned. We pre-loaded vaccinee data from the census on tablets to automatically populate the electronic form, shortening each vaccination interaction. During 40 days of vaccination, 45,368 people received a first OCV dose. Of those, 90.8% were documented to receive two doses. Nightly analysis of community coverage each day informed the next day's vaccination strategy. Toward the end of each phase, we generated case-finding reports allowing us to specifically identify those who had not yet been vaccinated. The tablets' GPS capability allowed us to map vaccine posts, population size and vaccine coverage, providing deeper understanding of the reach of the campaign. The tablets withstood natural elements and battery life was sufficient for daily use (external batteries were used as backup). The hardware and software were user-friendly enough for use by high school-educated staff. Though mHealth solutions require up-front financial investment and training, they reduce the need for manual data entry and paper forms, which are costly and increase the risk of error. The use of mHealth allowed accurate, fast and high quality data collection and a targeted vaccination strategy in an OCV campaign in rural Haiti.

526

PAPER TEST CARDS FOR FAST FIELD SCREENING OF SUBSTANDARD AND FALSIFIED PHARMACEUTICALS

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Substandard and falsified pharmaceuticals are a growing global health concern rooted in the information asymmetry between sellers and buyers. This presentation describes a novel approach to bridging this information gap by allowing rapid chemical analysis of pharmaceutical dosage forms without requiring laboratory facilities or instrumentation. We have devised an inexpensive and easy to use paper test card for rapid field screening of some common antibiotics and TB medications. The cards are printed with different reaction areas and loaded with chemical reagents; the cost of manufacture is under $US 0.50 per card. To use the test card, a pill is swiped over the reaction areas and the bottom edge is dipped into water. The capillary flow of water through the device activates different reagents and transports them to the drug sample. The test results appear as a color "bar code" that indicates the presence or absence of specific functional groups and compounds; the results can either be read by eye or by software that evaluates a cell-phone image of the test card. In a double-blinded laboratory validation, the test cards detected the active pharmaceutical ingredients (APIs) ampicillin, amoxycillin, acetaminophen, ethambutol, isoniazid, pyrazinamide, and rifampicin with high sensitivity and selectivity. Excipients such as starch, chalk, and talc were accurately detected, along with formulations that included substitute APIs or adulterants. This presentation will focus on a new version of the paper test card that can quantify APIs in solid dosage forms using colorimetric, titrimetric, and ratiometric approaches. Data will be presented on the limit of detection and limit of quantification of beta lactam antibiotics in formulations cut with various excipients, and discuss the scale of a regional post-market testing program that would be necessary for reliable detection of substandard or falsified drugs.

527

LINKING HUMAN AND ANIMAL HEALTH: A POPULATION BASED ANIMAL SYNDROMIC SURVEILLANCE STUDY

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Livestock production is the main source of livelihood for over 70% of the rural population in Sub-Saharan Africa. In smallholder production system, livestock are kept in close proximity to humans sharing same environments, increasing probability of zoonotic pathogen transmission. However there is a dearth of data on the socio-economic impact diseases have on small-scale animal ownership, and the impact zoonotic diseases have on human health. To remedy this, a study conducting a simultaneous multi-year syndromic surveillance in humans and their animals in 1600 rural households in Western Kenya is being conducted. Each of the study household is visited at least bi-weekly and data on four human syndromes; fever, jaundice, diarrhoea and respiratory illness (cough, pneumonia), and 9 animal syndromes; respiratory, death, reproductive, musculoskeletal, nervous, digestive, skin disorders, udder disorders, and urogenital syndromes collected in cattle, sheep, goats and chicken are collected. Additionally, a comprehensive socio-economic survey is conducted in each of the 1600 households quarterly. Preliminary results show 80% of the study households own cattle, 88% own chicken, 62% own goats and 39% own sheep. Digestive syndromes, mainly diarrheas are the most common syndromes observed in cattle, goats and sheep, and account for about 50% of the livestock syndromes. From these data, we will determine if disease/syndromes in humans and animals cluster in certain households and consequently determine factors associated with high disease burdens. Animal syndromes with the greatest economic impact on the human health and human syndromes impacting on animal production will be identified. This study provides a unique dataset directly linking human and animal health, and socio-economic status at the household level. Such data will increase our understanding of the health implications of livestock keeping, and provide information vital to policy makers in setting priority and strategy on integrated human-animal disease control.

528

EVALUATION OF CHROMATOGRAPHY PAPER AS A LOW-COST MEDIUM FOR ANEMIA DIAGNOSIS

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Anemia affects a quarter of the world's population, and a lack of appropriate diagnostic tools often prevents treatment in low-resource settings. Though the HemoCue 201+ is an appropriate device for diagnosing anemia in low-resource settings, the high cost of disposables ($0.99/test in Malawi) limits its availability. The low-cost WHO Hemoglobin Color Scale system ($0.02/test in Malawi) suffers from low accuracy. To address these concerns, we have developed a method that uses spectrophotometric measurement of blood spotted on chromatography paper as an accurate, low-cost (<$0.01/test) alternative to these methods. We optimized impregnating paper with chemicals to lyse red blood cells, paper type, drying time, wavelengths measured, and sensitivity to variations in volume of blood by using pipettes to apply blood to the paper and a laboratory spectrometer to take measurements. Lysing the blood

cells with sodium deoxycholate dried in Whatman Chr4 chromatography paper gave repeatable results, and the absorbance difference between 528 nm and 656 nm was stable over time in measurements taken up to 10 min. after sample preparation. The method was insensitive to the amount of blood spotted on the paper over the range of 5 µL to 25 µL. We created a low-cost, handheld reader to measure the transmission of paper cuvettes at these optimal wavelengths. Training and validating this device in a laboratory setting with patient samples on the handheld reader showed that the method is accurate to within 2 g/dL of the HemoCue device for 95% of samples. The measurement takes 6 seconds and can be performed at the patient's bedside. Field trials are planned for the summer of 2013 to evaluate our method in pediatric patients at the Queen Elizabeth Central Hospital in Blantyre, Malawi (n = 70) and in pregnant women at health clinics in Ventanilla and Cusco, Peru (n = 200).

529

MOSQUITOES MEET MICROFLUIDICS - HIGH-THROUGHPUT MICROFLUIDIC TOOLS TO STUDY FIELD ECOLOGY OF INSECT-BORNE INFECTIOUS DISEASES

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Just as high-throughput tools and measurements at single-cell or single-molecule resolution have impacted our fundamental understanding in biology and medicine, physics-based precision measurement tools can also be applied to study field ecology of infectious diseases. We propose a novel high-throughput ultra-low-cost microfluidic tool to enable large-scale ecological measurements in wild insect vector and pathogen populations. The proposed microfluidic device is a 2D paper-based matrix of collection pockets, each sized to isolate an individual insect bite. The device is baited and placed in a field site to collect saliva samples when bitten by insect vectors. After collecting bite samples, reagent is supplied and the chip thermally cycled to detect bite locations and identify bites containing pathogens of interest, using multiplexed Taq-Man qRT-PCR. This technique is applicable to all diseases that are transmitted via saliva of an insect vector. Current work on the device uses the *Culex pipiens* vector and West Nile virus as a model system. We have collected discrete salivary droplets preserved in a 2D matrix by allowing *Cx. pipiens* mosquitoes to probe agarose gels. A 234bp fragment of the Ace2 gene specific to *Cx. pipiens* was amplified from these droplets through PCR. We have constructed a paper-based microfluidic device that can process 20000+ nanolitre-volume reactions on a 75mm by 25mm area (equivalent to a microscope slide), reducing reagent costs by an estimated 50,000 times. With this new technique, spatial and temporal population data at single-vector resolution can be collected anywhere in the world for monitoring malaria, dengue, West Nile Encephalitis, or any other mosquito borne diseases. This technique can be used to quantitatively measure distribution of pathogens in a vector population, for a better understanding of vector-pathogen interactions and population dynamics at scales never before possible. The device presents a low-cost, scalable solution to enable large-scale vector screening efforts worldwide. This can enable the scientific community to explore new frontiers in insect-borne infectious disease research, such as automated construction of high-resolution vector surveillance maps around the world, understanding the influence of climate and seasonal factors, or application of molecular techniques to understand evolution of pathogens or insecticide resistance in real world settings.

530

A GEOSPATIAL ANALYSIS OF HEALTH CARE ACCESSIBILITY IN KENYAN HIGHLANDS

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In malaria-endemic, limited-resource regions, such as the western Kenyan highlands, seeking and having access to appropriate anti-malarial treatment is crucial. An important determinant of health-seeking behavior is accessibility to health care services. People living within 1 hour (of travel time) of a health care facility are considered by the WHO, to have health care access. We evaluated affordability and availability in determining health care access and health-seeking behavior. The inverse relationship between distance to facility and use of health care services has been well established, but has largely been determined by Euclidean distance. As utilization is affected by multiple factors, we designed a more comprehensive measure by integrating opinions and habitudes from 863 consumers, 15 health care facilities, and 135 retailers such as chemists or general shopkeepers. A spatial analysis of normative walking time from participants' households to their nearest available healthcare facility was used to evaluate health-seeking behavior based on actual travel time (vs Euclidean distance or perceived travel time). In spite of living less than 1 hour of travel time from a hospital or clinic (maximum calculated time: 46 minutes), 34% of participants did not exclusively choose to seek treatment from these facilities. Patients consistently overestimated the amount of time it would take to walk to the nearest health facility. This is a barrier to self-efficacy and presents an intangible hindrance to positive health-seeking behavior. Of those who sought treatment exclusively from commercial facilities, 25% did so despite needing to travel for longer periods of time in order to reach them. Surveys of area health care facilities indicated that 40% of them exhausted their stores of artemisinin-based anti-malarials at least 1 or more times per month. This lack of consistent access to appropriate medications may indicate why patients choose to seek treatment from commercial facilities instead. About 10% of the patients indicated that they take no action upon malaria symptom onset; however, patients' perception of affordability was not associated with the decision to seek treatment thus signaling a need for increased education and outreach in these rural communities.

531

STUDY *IN VITRO* OF *TAENIA SOLIUM* POST ONCOSPHERAL STAGE

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Taenia solium is a parasite that causes neurocysticercosis (NCC) in humans. After the *T. solium* oncosphere enters the brain, it develops into a cysticerci. As this happens, the parasite produces a variety of molecules, which modulate the host immune response in order to avoid parasite destruction. The stage between oncosphere and cysticerci is the post oncospheral (PO) stage, which has not yet been characterized or studied. Study of the PO stage is important as proteins released during the stage could be used to improve diagnosis or provide new targets for vaccine development. For this reason, the objective of this work is to study the morphology and expression of total proteins during the PO stage. In vitro-hatched oncospheres of *T. solium*, prepared by the sodium hypochlorite method and activated using artificial intestinal fluid, were incubated on human intestinal monolayer cell. The parasites were collected at 1, 15, 30, and 60 days of incubation. The morphology was directly observed by microscopy. The proteins expressed in the oncosphere, PO, and cysticerci stage were compared by immunoblot. On day one of incubation, the activated oncosphere was, on average, 20 µm in diameter. The size of PO stage increased in relationship to the day of incubation, reaching up to 2500 µm in diameter at 60 days of incubation. Additionally, the number of cells and the thickness of oncospheral tegument also increased. When inoculated into the brain of a rat, the PO stage became cysticerci. By immunoblot, the PO stage has some proteins from both the oncosphere

and cysticerci stages. However, the expression of the oncosphere proteins decreases and the expression of the cysticerci proteins increases when the PO stage increases in size. It is possible that PO stage changes the expression of these proteins to modulate the host immune response and avoid its own destruction. When we tested a pool of sera from patients with NCC using proteins from the PO stage, two bands reacted strongly in immunoblot. These proteins could be used to diagnose patients with NCC in a noninvasive and relatively simple manner. This is the first time *T. solium* PO stage has been characterized *in vitro* and the discovered protein expression and parasite growth behavior could be used for diagnosis and further treatment development.

532

EFFICACY OF A TSOL18/ISA206V VACCINE CANDIDATE AGAINST PORCINE CYSTICERCOSIS

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Taenia solium is a zoonotic parasitic disease that affects pigs and humans. The adult stage lives in the small intestine of humans; eggs are released to the environment through human feces. Pigs are infected after ingesting human feces containing eggs or proglottids and developed the larval stage (cysticercosis) mainly in muscle tissues. If a human is accidentally infect with eggs by eating contaminated food or water, he/she would develop neurocysticercosis, the major cause of acquired epilepsy in developing countries. It has been postulated that the disease could be prevented and controlled by pig vaccination; however, current vaccine candidates, such as Tsol18 recombinant, demand elevated costs of preparation. Therefore, finding an affordable and effective vaccine candidate will be critical for disease elimination efforts. The present study aimed to test the efficacy for a similar recombinant vaccine candidate using a different adjuvant (TSOL18/ISA206V) sponsored by GALVmed, a not-for-profit global alliance which aimed to improve human lives by applying livestock vaccines. A randomized controlled trial (n=49) was designed and TSOL18/ISA206V vaccine efficacy was compared to previously reported effective vaccine TSOL18/QuilA (Australian vaccine). Vaccine efficacy was measured by vaccination followed by oral challenge of *T. solium* proglottid and evaluated by carefully carcass examination (gold standard). The TSOL18/ISA206V vaccine candidate presented high vaccine efficacy (83.3%) compared with the control group and showed no difference when comparing its results with TSOL18/QuilA (Australian vaccine) or the same vaccine with QuilA as the adjuvant. The presence of TSOL18-specific antibodies was assessed by ELISA test showing that all of the vaccinated pigs were positive after the second vaccination. TSOL18/ISA206V vaccine is a promising candidate for a marketable vaccine against porcine Cysticercosis. Further studies should be carried out to evaluate other aspect of the immunogenicity of TSOL18/ISA206V vaccine and its effectiveness and efficiency.

533

NEUROCYSTICERCOSIS TREATMENT USING RAT MODEL WITH *TAENIA SOLIUM*

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Neurocysticercosis (NCC) is caused by *Taenia solium* larvae infection of the central nervous system (CNS). NCC is the leading cause of acquired epilepsy and seizure conditions worldwide, especially in developing countries where pigs are raised and pork is consumed. In this study, we used a novel rat NCC model infected intracranial with activated *T. solium* oncospheres to evaluate and compare intraparenchymal and extraparenchymal infections, and to determine whether the model is beneficial for evaluating NCC treatment schemes. The First objective was to compare intraparenchymal, and extraparenchymal infections using different numbers of activated oncospheres (30, 60, 90, 180, 360, 720). The animals were sacrificed four months after infection. We found that the location of the infection was a factor to developing cysticercus. The proportion of animals that developed cysticercus in the brain was higher in the intraparenchymal group than in the extraparenchymal group. We also found that the number of cysticercus per brain increased with the number of oncospheres. The second objective was to assess the efficacy of Praziquantel and Albendazole to treat NCC. Rats infected with *T. solium* oncospheres were treated four months after infection with Praziquantel (75mg/Kg/day) plus albendazole (15mg/Kg/day) during 3 days, followed by 7 days of treatment with albendazole (15mg/Kg/day) only. The animals were sacrificed one month after treatment. Using histochemical by H&E, we observed that the cysticercus from treated animals demonstrated a partially degenerated cyst, with not whole destroyed cyst wall with high inflammatory response surrounded by a thick layer of collagen type I. The control group (infected, untreated rats) showed cyst viable parasite (scolex) with intact vesicular cyst wall, minimal to moderate host reaction (inflammatory response) with only a thin layer of collagen type I, which was either intact or mingled with inflammatory infiltrate. These results imply that the rat NCC model could be a beneficial model for understanding the progression of NCC in humans, and provide useful information for treatment.

534

THE MONETARY BURDEN OF CYSTICERCOSIS IN MEXICO

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Taenia solium cysticercosis is a major public health and agricultural problem in many developing countries where health education, sanitation, and meat inspection infrastructure are insufficient. Cysticercosis affects both human and animal health and has important economic consequences. Very few studies have been conducted to evaluate the monetary burden of cysticercosis. This study provides the first estimate of the monetary burden of cysticercosis in Mexico. The total monetary burden of cysticercosis, in Mexico, was estimated by assessing costs associated with infection of both humans and pigs. The cost of neurocysticercosis (NCC), in humans, took into consideration losses due to NCC-associated epilepsy and NCC-associated severe chronic headaches. Epidemiologic and

economic data were obtained from the published literature, government reports, and interviews and chart reviews of NCC patients treated at two neurological referral hospitals located in Mexico City, Mexico. Latin hypercube sampling methods were employed to sample the distributions of uncertain parameters and to estimate 95% credible regions (95% CRs). The overall monetary burden of human NCC, for Mexico, was estimated at U.S.$71,267,032(95% CR U.S.$44,470,935 - U.S.$103,235,886) per year, of which 45% was attributed to individuals with NCC-associated epilepsy, 39% was attributed to individuals with NCC-associated severe chronic headaches, and the remainder was attributed to NCC patients with both clinical manifestations. An additional U.S.$23,078,764 (95% CR U.S.$8,020,568 - U.S.$39,757,504) was estimated to be lost due to cysticercosis in the Mexican pig population annually. This study suggests that *T. solium* cysticercosis continues to result in considerable monetary losses to Mexico.

535

MANAGEMENT OF CYSTIC ECHINOCOCCOSIS: THIRTY YEAR EXPERIENCE IN A SINGLE REFERRAL CENTER IN NORTHERN ITALY

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Cystic echinococcosis (CE) is a chronic, complex and neglected disease. In humans, its clinical spectrum ranges from asymptomatic infection to severe, occasionally even fatal disease. Four management options exist: surgery, percutaneous techniques and drug treatment for active cysts, and watch and wait for uncomplicated inactive cysts. However the four options have never been properly evaluated and compared, and the evidence base for clinical decision making is still limited. In this context, we describe our experience with clinical management of CE in a single referral center in North Italy over a time span of 30 years and look at lessons learned. Patients referred for confirmed or suspected CE from 1982-2012 were included. Data were available for 1022 patients and 1295 cysts. CE was confirmed in 695 patients (881 cysts), while the remaining 327 were non-parasitic cystic lesions. Patients with CE related symptoms were 418 while 277 (40%) were asymptomatic. Among 440 patients diagnosed with CE and monitored over time, 297 (67%) have been treated, while 143 (33%) have been managed expectantly. Of the 695 CE patients, 440 (63%) have been followed-up for a mean period of five years. Foreign patients (203, 29% of the total number) were mostly immigrants from the highly endemic areas of Northern Africa and Eastern Europe, and their number has steadily increased over the years. In our cohort, almost a third of the patients were managed expectantly, thus saving resources and avoiding unnecessary treatments. Ruling out non parasitic cystic lesion is an important part of diagnostic workup. The number of immigrants with CE has steadily increased in the last 10 years. CE patients should be managed in referral centers until guidelines on treatment with a strong evidence base become available.

536

CENTRE-BASED CLINICAL MANAGEMENT OF CYSTIC ECHINOCOCCOSIS

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Neglected infectious disease (NIDs) play an increasing role in non-endemic countries at the current level of global mobility. Cystic echinococcosis (CE) is one of the world's most neglected NIDs. CE lesions, predominantly in the liver and lungs, develop silently over long periods of time until complications suddenly precipitate. In high-income countries mostly immigrants from CE-endemic areas are affected and health services are, as a rule, not experienced to diagnose, stage and manage CE patients appropriately. The setting and the impact of the interdisciplinary CE Centre at Heidelberg University Hospital is presented where infectious disease / tropical medicine physicians, radiologists, abdominal and thoracic surgeons, gastroenterologists and parasitologists work very closely together to stage CE patients (ultrasound-based cyst classification) and to tailor currently available treatment options (medical treatment with albendazole, percutaneous cyst-sterilization techniques, surgery and 'watch and wait') to the needs of the individual patient.

537

IMMUNOBLOTTING WITH HUMAN ANTIGEN CORRELATES WITH CYST STAGE IN PATIENTS WITH CYSTIC ECHINOCOCCOSIS OF THE LIVER

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Cystic Echinococcosis (CE) is a zoonosis caused by infection with the larval stage of *Echinococcus granulosus*. Although the natural history of cyst development is not completely known, they likely pass through several stages, from active to inactive forms. The diagnosis of hepatic CE is based on ultrasonography, confirmed by a combination of immunodiagnostic tests. These, however, lack standardization and have low sensitivity and specificity. In addition, no serological marker is currently available which correlates with cyst viability or the ability to predict the evolution of a cyst, implying the need for years-long patient follow-up after treatment. We present a preliminary characterization of the performances of an immunoblotting (IB) test based on human hydatid cyst fluid (HCF) with particular regard to its ability to distinguish between cyst stages. Patients with active cysts (CE1 and CE2) responded differently to HCF: while patients with CE2 cysts consistently recognized subunits of CE major antigen AgB, these were inconsistently recognized by sera from CE1 patients. Patients with inactive cysts (CE4 and CE5) did not recognize any specific band. Finally, patients with transitional cysts (CE3a and CE3b) recognized subunits of AgB and Ag5. Most importantly, the experimental IB allowed in many cases to discriminate between CE3a and CE3b, known to have different viability profiles. In an attempt to assess whether the experimental IB could detect early changes in cyst viability, we tested it on sera from one patient at time-points where the hepatic CE cyst passed from active (CE1) to transitional (CE3a) to inactive (CE4) stages after albendazole treatment. We observed a rapid change in band pattern recognition. These findings strongly support the hypothesis that different antigens are expressed by different cyst stages, whose recognition might be useful in clinical practice to correctly define cyst viability and open new opportunities to develop diagnostic tools that could guide clinical decision-making and shorten patient follow-up.

538

REACTIVE CASE DETECTION FOR MALARIA ELIMINATION IN SWAZILAND: FACTORS ASSOCIATED WITH THE DETECTION OF SECONDARY *PLASMODIUM FALCIPARUM* INFECTION

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Reactive case detection (RACD), the screening of household members and neighbors of passively detected malaria cases for infection, is recommended for malaria elimination but there is little evidence to guide

practice. We performed a prospective surveillance study in Swaziland to identify factors related to the index case or individuals screened associated with the detection of secondary cases. We also compared RDT to a molecular method, loop-mediated isothermal amplification (LAMP), for the detection of secondary cases. RDT and/or microscopy confirmed index cases reported from 294 health facilities in Swaziland were targeted for follow-up. If there was potential local acquisition (area receptive to malaria transmission or no travel history), family and neighbors residing within a 1 km radius were targeted for RACD. We collected dried blood spots (DBS), GPS coordinates, and information on demographics, travel, vector control, housing, season, area receptivity, response time and coverage of RACD, and index case clinical characteristics. Family and neighbors testing positive by RDT were referred for treatment; DBS from all subjects were tested by LAMP. Bivariate analyses of potential relationships between risk factors and secondary case detection by LAMP were performed using t-test or logistic regression. From 8/2012 to 4/2013, 165 index cases were identified; 56 qualified for and underwent RACD resulting in 1481 household members and neighbors screened. Secondary cases were more likely to occur when the index case was LAMP positive at follow-up (OR 6.9, 95% CI 1.3-36.8, median follow-up at 5 days, 95% CI 1-29), RACD was timely (within 4.9 days, 95% CI 2.7-8.8, vs. 11.6, 95% CI 8.3-16.1, p=0.009), and more subjects screened (mean 28 people, 95% CI 12-63, vs. 10, 95% CI 7-15, p=0.008). Among individuals screened, LAMP positivity was associated with travel outside Swaziland (OR 12, 95% CI 5.1-28.2) and closer distance to the index case (mean 39.8m, 95% CI 13.6-116.4, vs. 151.0m, 95% CI 134.6-169.4, p=0.001). To date, LAMP has detected 5 fold more infections than RDT (2.5%, 27/1093, LAMP positivity vs. 0.5%, 6/1093, RDT true positivity using LAMP as gold standard). Post-treatment LAMP positivity in index cases is likely due to gametocytes and points to a potential role for additional gametocidal agents to prevent onward transmission. The effectiveness of RACD to detect secondary infections can be improved using LAMP, optimizing response time, screening radius size, and target population.

539

HIGH PREVALENCE OF ASYMPTOMATIC MALARIA IN URBAN SETTINGS IN DOUALA, CAMEROON

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Malaria remains a major health problem in Cameroon with 38% of consultations and 24% of deaths. The negative economical impact of malaria has encouraged a new approach targeting companies with counseling and distribution of prevention kits for workers and their families. A cross sectional study was undertaken from October 2012 to February 2013 to collect preliminary data to assess the impact of the Exxon Mobil foundation control program in enterprises and communities in Douala, which consisted of indoor spraying and distribution of Long Lasting Impregnated Nets (LLINs). 2191 people in six communities and 829 in three enterprises were interviewed and screened with a mass diagnosis method based on malaria rapid blood test using pre-stained slides for fluorescence microscopy (CyScope®, Partec GmbH, Germany). Alongside, 783 children were also screened in five schools. All positive cases were treated immediately. A high prevalence of asymptomatic malaria was determined with a mean of 38,02% in the screened population of 3803 individuals. 45,47%, 40,48% and 24,49% of malaria tests were positive in schools, communities and companies respectively. Only 1% of the positive cases had fever. The prevalence in schools correlated with sanitation indices. The highest prevalence in communities was registered among children under five (42,22 %). Out of 2494 persons who responded to questionnaires, 1614 owned a LLIN. This group was less affected by malaria infection than those without nets, although the difference was not significant. (36,93% against 38,52%; X2 = 0,61, ddl = 1, P>0,05). The average coverage was 3,10 persons/net. The impact of malaria control initiatives can be better assessed with the use of mass diagnostic tools. Asymptomatic malaria is highly prevalent in Douala but coverage with LLINs is still insufficient. This situation makes malaria elimination difficult to envisage in endemic areas. However, malaria elimination can be foreseen if all detected cases are promptly treated and concomitantly protected from anopheles bites during the lifespan of gametocytes in persons with parasitaemia.

540

A 2012 DEMOGRAPHIC AND SEASONAL PROFILE OF HUMANS HOSTING THE MALARIA PARASITE RESERVOIR IN ZAMBIA: RESULTS FROM MASS SCREEN AND TREAT (MSAT) ACTIVITIES IN SOUTHERN PROVINCE AND IMPLICATIONS FOR ELIMINATION

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Control programs aiming for malaria elimination are tasked with clearing parasites from people and preventing transmission from mosquitos to humans. Documenting the human parasite reservoir and profiling demographic characteristics and seasonal variations can guide efforts to clear parasites from people and prevent transmission. Three rounds of MSAT in southern Zambia in 2012 provide this unique information. MSAT covering a population of approximately 90,000 residing in 4 districts was carried out during low transmission season (April to October). A rapid diagnostic test was administered to all individuals and infections were treated with artemether lumefantrine. District parasite prevalence during round 1 ranged from 3% to 44%; in round 2, from 5% to 22%; and in round 3, from 2% to 16%. Trends in parasite prevalence were highest across rounds among children age 5-14 years, and lowest among adults age 50+. Nearly half of the parasite reservoir consistently resides in children age 5-14. In comparison with population age structure, the reservoir disproportionately resides within this age group. Infants and individuals age 25 and above are disproportionately less likely to carry malaria infection. Results indicate need to target school age children, a finding in line with 2012 Malaria Indicator Survey (MIS) results indicating need to improve bed net usage among this age group. MIS results indicate that bed net usage is high until age 5 (57%), falls among school age children (age 5-9, 45%; age 10-19, 37%), and increases with age from age 20 to 49 (e.g. age 45-49, 61%). Another important characteristic of the parasite reservoir in this context is that it is largely characterized by afebrile infections. Infected individuals reporting recent fever ranged from 13% to 23% in round 1; 4% to 7% in round 2; and 2% to 8% in round 3. Afebrile infection is the norm across the lifespan, although older age (50+) is associated with higher likelihood of fever accompanying infection. These results highlight the importance of clearing parasites from asymptomatic (afebrile) people.

541

EFFECTS OF MALARIA CONTROL ON THE HEALTH FACILITY: CHANGES IN COSTS AND HOSPITALIZATIONS IN TWO HOSPITALS IN ZAMBIA

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The intensive scale-up of malaria control efforts in recent years has significantly reduced the malaria burden worldwide. There is little evidence, however, of the impact of malaria control on broader health systems, particularly at the health facility level. We present a pre-post comparison of hospital admissions and outpatient visits for malaria before and after the scale-up of malaria control by using retrospective, longitudinal facility-level data and patient record data covering the period 2003 to 2008 from two hospitals in Zambia's Southern Province. We also conducted costing analyses to estimate the costs of testing and treating malaria patients by year at both hospitals to determine changes in the total financial resources devoted to malaria admissions over time as malaria control is scaled up and inpatient malaria admissions decrease. Results show a substantial reduction in inpatient admissions and outpatient visits for malaria at both hospitals following the scale-up. The proportion of total hospital visits for malaria decreases over time in both facilities. At one hospital, malaria admissions account for 20% of total admissions for patients under-5 before malaria control scale up, compared to 1% after malaria is controlled. Hospital spending on malaria admissions also decreased with the expansion of malaria control. In one hospital, malaria accounted for 11% of total hospital spending before malaria control scale-up compared to less than 1% of hospital spending following malaria control. The study findings demonstrate that as malaria is better controlled in the catchment area facility-level resources used for malaria treatment also decline, potentially freeing resources for the treatment of other conditions.

542

RESPONSE TO ANTIMALARIAL THERAPY WITH ARTEMETHER-LUMEFANTRINE AMONG INFANTS RANDOMIZED TO THREE DIFFERENT ANTIMALARIAL CHEMOPREVENTION REGIMENS IN AN AREA OF HIGH TRANSMISSION INTENSITY

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The burden of malaria remains high for children in parts of Africa despite the use of insecticide treated bed nets (ITNs). Chemoprevention has the potential of reducing the malaria burden; however, limited data exist on the efficacy and safety of anti-malarial therapy in the setting of chemoprevention. A cohort of 400 infants aged 4-5 months were enrolled using convenience sampling in Tororo, Uganda, an area with perennial high transmission intensity, given an ITN, and followed for all their health care needs 7 d/wk. At 6 months of age, 393 infants were randomized using an open label design to one of four treatment arms; no therapy, monthly sulfadoxine-pyrimethamine (SP), daily trimethoprim-sulfamethoxazole (TS), or monthly dihydroartemisinin-piperaquine (DP). Study drugs were administered unsupervised at home until 24 months of age. Episodes of uncomplicated malaria were treated with artemether-lumefantrine (AL) and followed for 28 days. The risk of day 3 *parasitemia*, recurrent *parasitemia*, and adverse events was compared across chemoprevention arms using generalized estimating equations.767, 734, 618, and 368 episodes of malaria were treated in the no therapy, SP, TS, and DP arms, respectively. Only 21 of 2487 (0.8%) treatments were for complicated malaria (12 danger signs and 9 severe malaria). Following treatment for uncomplicated malaria with AL, 99.3% achieved parasite clearance by day 3 and the cumulative risk of recurrent *parasitemia* after 28 days was 46.0% and did not differ across the 4 chemoprevention arms (range 44.7-47.0%). Compared to the no therapy arm; the SP arm was associated with a higher risk of jaundice (1.5% vs. 0.1%) and a lower risk of diarrhea and vomiting; the TS arm was associated with a lower risk of cough, diarrhea, anorexia, and anemia; and the DP arm was associated with a higher risk of neutropenia (16.6% vs. 10.0%) and a lower risk of anemia. The risk of complicated malaria was very low in this cohort of infants living in a high transmission setting. Treatment of uncomplicated malaria with AL was associated with excellent parasite clearance and treatment efficacy and safety were not significantly influenced across the range of chemoprevention regimens.

543

IMMUNE PROTECTION AFTER MALARIA CEASED ON ISLANDS

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Re-infection is a major concern after malaria elimination. After weekly mass drug administration for nine weeks combined with vector control on the entire population (718) of Aneityum island in 1991, *Plasmodium falciparum* disappeared and *P. vivax* from 1996 onwards. Transmission interruption was sustained until a *P. vivax* epidemic was reported in January 2002. We aimed to assess age-specific malaria prevalence during this epidemic. Two cross-sectional malariometric surveys of the entire population of Aneityum were conducted six and ten months after the index cases. In July 2002 *P. vivax* infections were detected by microscopy in 22/759 individuals: 20/298 born after beginning the elimination programme in 1991, 2/126 between 1991 and 1982, and 0/339 before 1982. PCR diagnosis increased the infection number to 77 distributed amongst all age groups. Parasite prevalence was 12·1%, 16·7%, and 6·0%, respectively. In November a similar age pattern was found but with fewer infections: 6/746 and 39/741 by microscopy and PCR, respectively. All microscopy positive cases were PCR positive. Antibody responses to *P. vivax* were significantly less for individuals born after 1991 than for older age groups. A remarkably low genotype diversity of immune target antigen genes *Pvmsp1* and *Pvcsp* observed in Aneityum (h= 0·15) when

compared with the other islands (h= 0·89–1·0) suggests a recent parasite re-introduction was linked with malaria resurgence. Acquired immunity against *P. vivax* persists after malaria exposure has ceased and appears to protect individuals born before elimination from clinical disease. The immunity does not prevent infection per se but suppresses erythrocytic stage infection to sub-microscopic levels. The stable and limited antigen SNPs on islands may likely underline anti-malarial immune protection against reinfections in Aneityum adults.Protection is required to prevent young populations from clinical diseases, but interventions including all populations remain critical to sustain malaria elimination.

544

GENETIC SURVEILLANCE DETECTS BOTH CLONAL AND EPIDEMIC TRANSMISSION OF MALARIA FOLLOWING ENHANCED INTERVENTION IN THIÈS, SENEGAL

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Using whole genome sequencing information we developed a molecular barcode tool that queries independent single nucleotide polymorphisms from the *Plasmodium falciparum* genome Genotyping data obtained from screening patients with mild uncomplicated malaria seeking treatment at a clinic in Thiès, Senegal from 2006 to 2011 revealed an increasing frequency of infections caused by genetically identical parasite strains representing 10% of the population in 2006 and more than 50% of the sampled population in 2011 that coincided with growing deployment of malaria control interventions and decreased malaria deaths. Several of these parasite genotypes persisted clonally across different transmission seasons. We also observed an increase in the frequency of genetically identical parasite strains corresponded with a decreased probability of multiple infections. We performed a pilot survey of asymptomatic individuals from the same clinic catchment site and observed a similar frequency in the number of shared barcodes among this population indicating that specific parasite types were not specifically associated with illness in the passive case detection population. We are addressing whether immunity plays a role by looking at the distribution across the age spectrum and are investigating whether patients harboring parasites with shared barcodes have a higher gametocyte carriage rate that may imply they are more easily transmitted in the population. Analysis of these trends support evidence of both clonal and epidemic population structures. These data provide the first evidence of clonal parasite populations in Africa emerging after deployment of substantive malaria-reducing efforts including bednet distribution, use of rapid diagnostic tests for case detection, and treatment with artemisinin combination therapy. We hypothesize that reduced malaria transmission results in decreased outcrossing in the mosquito midgut that results in emergence of clonal parasite types when transmission becomes sufficiently reduced. This implies that genetic surveillance with genotyping tools that assess these changes in parasite population structure can be used to evaluate the effectiveness of disease control strategies and assist a rational global malaria eradication campaign.

545

CASE INVESTIGATION AND REACTIVE INFECTION DETECTION FOR MALARIA ELIMINATION IN NORTHERN SENEGAL

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A malaria case investigation program was piloted for 12 weeks in 2012 in Richard Toll district of northern Senegal. Malaria infections (N=110) were identified through facility-based passive case detection and investigated within 3 days. RDTs and a brief questionnaire were administered to 5520 individuals (average of 50 contacts per index case) living within the index case compound or within 5 neighboring compounds. In comparison with family and neighbors, index cases were more likely to be male, age 15-49, employed outside of the home, and to report recent travel. Twenty-three (0.4%) of the family/neighbors were RDT positive. Potential risk factors for infection among family and neighbors were examined including: sex, age, occupation, travel history, bed net usage, and residence (index vs. neighboring compound). Adjusting for all factors, risk of infection was associated with recent travel and residence in the index case household. RDT positivity was notably high among people with recent travel to Dakar (10.5%) or other regions in Senegal (33.3%) as compared with those who did not report such travel (0.2%). Recent fever among RDT-positive family and neighbor contacts was uncommon (30%). We examined possible screening criteria that would optimize the efficiency of contact investigations in this population. Rather than testing all 5520 people in index and neighboring compounds to identify 23 infections, if testing was targeted to all people in the index compound and only neighbors who report recent travel or fever, only 1173 individuals would be tested to identify 20 of the infections. The remaining 3 cases not identified by screening for recent fever or travel would have been identified by including screening questions regarding boarding at school outside of the region. Expanding and optimizing case investigation with specific targeted testing and treatment of at-risk contacts can facilitate continued progress towards malaria elimination in northern Senegal.

546

SPATIO-TEMPORAL PATTERNS ASSOCIATED WITH DELIVERY OF A MASS SCREEN AND TREATMENT CAMPAIGN IN SOUTHERN ZAMBIA: IMPLICATIONS FOR MODELING SYSTEM PARAMETERS

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The Zambia National Malaria Control Program has successfully scaled-up recommended malaria control interventions over the past decade and is pursuing alternative methods to further reduce malaria transmission including community-targeted, parasite reservoir reduction strategies. During the 2012 dry-season (June-November) in Southern Zambia, three large-scale mass-screen-and-treat (MSAT) rounds were undertaken with rapid diagnostics tests (RDT) and the anti-malarial drug, artemether-lumefantrine. Randomized health facility catchment populations were visited at their homes in a full community census and, following individual consent, were administered rapid diagnostic tests and RDT-test

positive individuals were provided treatment according to national policy recommendations. Using the surveillance data collected during these activities, we analyze spatio-temporal patterns and extract parameters relevant to modeling the system and its response to intervention campaigns. These include data-driven estimates of programmatic coverage rates, the extent of internal migration within the study area, the underlying age- and exposure-specific immunity, and the potential effects of heterogeneous biting and non-compliant usage of distributed drugs and ITNs on re-infection risk. As malaria control and elimination efforts progress, optimizing the combination of prevention and treatment strategies for delivery at community level are essential to reduce the transmission potential among those asymptomatic carriers.

547

HEALTH FACILITY INCIDENCE OF SEVERE MALARIA AND PNEUMONIA IN CHILDREN UNDER FIVE, FOLLOWING A RANDOMIZED INTEGRATED COMMUNITY TRIAL IN EASTERN UGANDA

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In Africa, malaria and pneumonia are among the leading killers of children under five. The overlap of clinical presentation between the two diseases poses a challenge especially for clinicians with limited diagnostic tools. The Integrated Community Case Management (ICCM) of Childhood Malaria, Pneumonia and Diarrhea has been adopted by many African countries including Uganda. A randomized controlled community trial was conducted in Eastern Uganda, in which integrated care for malaria and pneumonia was provided in intervention and control villages. Under five children in intervention villages received Coartem (CoA) for the treatment of malaria and amoxicillin (Amox) for pneumonia while children in control villages only received CoA for malaria. This post-intervention study assessed the incidence of severe malaria and pneumonia at health facilities in the study area, before and after intervention. Health facility incidence of severe malaria and pneumonia was used as a proxy measure of severe incidences for the two diseases at community level. Health facility records over a 5 year review period (n=10,236) on severe malaria and pneumonia in children under five were abstracted using a health facility tool based on WHO clinical classification for severe malaria and pneumonia. Their incidences were compared before and after study intervention, and between intervention and control study villages. The incidence of severe malaria in study villages was 943/20770 (4.5%) before intervention, compared to 999/22674 (4.4%) post study intervention (OR=0.97, 95% CI [0.89 -1.06] p value 0.52). Incidence of severe pneumonia was 32/2223 (1.4%) before intervention compared to 53/2714 (2.0%) after intervention (OR=1.36, 95% CI [0.85 -2.16], p value 0.17). After intervention, the incidence of severe malaria from intervention villages was 4.4% compared with 17% from non-study villages (OR=0.26, 95% CI (0.24-0.28) P value <0.001). Following intervention, the incidence of severe pneumonia was 2% intervention villages compared with 22.7% for non-study villages (OR=0.47, 95% CI (0.39-0.57) P value<0.001). Overall, the proportion of children presenting with severe morbidity due to malaria and pneumonia was much lower in study villages after intervention, compared to non-study villages.

548

EVALUATION OF THE TOLERANCE OF SULFADOXINE-PYRIMÉTHAMINE + AMODIAQUINE COMBINATION IN SEASONAL MALARIA CHEMOPREVENTION (SMC) COMBINED WITH HOME BASED MANAGEMENT (HMM) IN CHILDREN UNDER 10 YEARS IN SENEGAL

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Seasonal Malaria Chemoprevention (SMC) is an important tool for malaria prevention in children in sub-Saharan areas where transmission is intense and seasonal. It showed superior efficacy to 70% with AQ + SP combinations. Nevertheless, the data related to the tolerance of the products remain low and access to health facilities is an obstacle to the notification, the objective of this study was to assess the safety of SP¨+AQ when administered by Community Health Workers (CHWs) and home care Providers living in the same villages as mothers who report adverse events (AEs). The study was conducted in the health district of Saraya located in the south-eastern of Senegal. The CHWs/Providers, community supervisors and qualified district staff had been trained in recognition of AEs related to SP and AQ. During the last two cycles of the intervention a child sample was selected and their mothers visited the 4th day to assess the proportion of children who vomited the second and third doses administered at home. Every two weeks supervisions were conducted to collect tools. In total, 40 officers were trained and 33000 doses administered between July and November 2011, 29 notifications were recorded, including 14 due to SMC. Vomiting was more reported with an incidence of 37.9% and 1 case of rash was recorded. Most of rejection of drugs was notified causing a decline in coverage in a village. This decrease was corrected through social mobilization. A decline in notifications was noted over time. No case of serious AEs was recorded. The involvement of CHWs/Providers is important in the notification and the possible detection of severe cases for their proximity to children receiving SMC under implementation.

549

CLUSTER RANDOMIZED TRIAL OF AN INNOVATIVE PAY-FOR-PERFORMANCE (P4P) STRATEGY TO IMPROVE DIAGNOSIS AND TREATMENT OF MALARIA IN WESTERN KENYA

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In rural health facilities without malaria diagnostic tests, as many as 90% of febrile patients receive an antimalarial. Moreover, in facilities with testing services, 40-80% of patients with a negative test receive an antimalarial. This practice leaves the true cause of fever untreated, accelerates the spread of drug resistance, and wastes costly drugs. Pay-For-Performance (P4P) programs have generated interest as a potential mechanism to improve health service delivery and accountability. However, there has been little experimental evidence to assess the effectiveness of P4P programs in developing countries. We describe a cluster-randomized controlled trial underway in 18 health centers in western Kenya testing an innovative incentive strategy to improve diagnosis and treatment of malaria. The incentive scheme promotes adherence to WHO guidelines for laboratory confirmation of malaria before treatment. There are three important innovations to this study: the behavior being incentivized is quality of care rather than volume of service; the incentives are applied at the facility rather than individual level, thus benefiting overall facility infrastructure and performance; and the incentives are designed to be budget-neutral. Following clinical and laboratory training and

establishment of a monthly EQA for malaria microscopy, the percent of malaria cases confirmed by laboratory diagnosis increased from 25% to 52% while slide positivity decreased from 30% to 20%. The mean sensitivity and specificity for a six-month period across the 18 facilities was 98% and 85%, respectively. Monthly artemether-lumefantrine consumption decreased by 34% between the period prior to the training and the period following the training. We will also discuss the effect of the intervention on malaria testing and prescription practices, as well as projected cost savings, after one year of incentives. This study will demonstrate whether facility rather than individual incentives are compelling enough to improve case management, and whether these incentives lead to cost-savings due to reduced drug consumption.

550

USE OF INSECTICIDE QUANTIFICATION KITS (IQK) TO INVESTIGATE THE QUALITY OF SPRAYING AND DECAY RATE OF BENDIOCARB ON DIFFERENT WALL SURFACES IN KAGERA REGION, TANZANIA

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Bendiocarb was introduced for Indoor Residual Spraying (IRS) in Tanzania in 2012 as part of insecticide resistance mitigation. This study aimed to monitor insecticide concentration to assess 1) intra-operational IRS coverage and quality of spraying and 2) decay rate of insecticide on different wall surfaces. The study was conducted in Muleba and Karagwe districts. Wall substrate samples were obtained by scratching wall surfaces using a scalpel and collecting it in eppendorf tubes. To assess intra-operational IRS coverage and quality of spraying, 102 houses were randomly selected. A total of 510 samples (218 in Muleba and 292 in Karagwe) were obtained for IRS coverage and intra-spraying quality assessments. To investigate decay rate, 30 houses in Karagwe with recommended concentration at baseline were selected. The wall substrates included: burnt bricks (five); cement plastered (three); mud wall (twenty); and lime plastered walls (two). Follow-up samples were collected on monthly basis for over a period of five months. Laboratory testing of insecticide was done using the Carbamate Insecticide Quantification Kit (IQKTM [Innovative Vector Control Consortium, www.ivcc.com]). The IQK assay is based on inhibition of the activity of recombinant acetylcholinestarase (AChE) by bendiocarb which is dependent on the concentration of insecticide. Of the 510 samples, 89.4%(95% confidence interval [CI]86.4-91.9) met the WHO recommended concentration for IRS coverage. The proportion of houses meeting WHO standards for IRS coverage varied between Muleba (96.3%) and Karagwe (84.2%) (p-value<0.001). Bendiocarb decay follow-up in Karagwe showed that the proportion of houses with recommended concentration (between 100 - 400 mg/m2) declined from 93.3%, 92.6% and 73.1% at months one, two, and three post IRS, respectively (ptrend=0.03). The acceleration of decay increased in fourth and fifth month post IRS with WHO standards met in only 30.1% and 7.7% of houses, respectively. All house surfaces meeting WHO standards at month five were made of burnt brick walls. IQK is an important tool for assessing IRS coverage and quality of spraying, and can monitor insecticide decay over time to establish the right time to conduct a new spray cycle.

551

IMPROVING GLOBAL FUND PROGRAMMATIC INDICATOR PERFORMANCE - KENYA, 2012

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Kenya received a Global Fund (GF) Round 10 grant to strengthen national malaria control activities. GF disbursed $7.8 million, including $5.4 million for commodities, to Kenya in 2012. In October 2012, the overall grant performance was rated "unacceptable" (i.e., C rating). We subsequently implemented two strategies to address programmatic performance. We integrated malaria commodities data reporting, including rapid diagnostic test and artemether-lumefantrine treatment, into the routine District Health Information System (DHIS2) to ensure all malaria indicator data was reported monthly in a single system. The President's Malaria Initiative also supported a modest one-time reimbursement (up to $175) for facilitated supervision expenses incurred by district pharmacists to collect malaria commodities data from non-reporting facilities via telephone, short message service or visits and enter the data into DHIS2. Reporting of malaria commodities data by health facilities via the legacy Logistic Management Information System averaged 40% (range 37-45%) per month between January-September 2012. Reporting via DHIS2 increased to 72% (range 71-72%) per month between October-December 2012. The GF core programmatic indicator, the percent of target reached for number of people treated appropriately for malaria, averaged 40% (range 25-56%) per quarter between January-September 2012. The GF core programmatic indicator increased to 92% for the last quarter from October-December 2012. Ninety of 285 (32%) district pharmacists requested reimbursement; reimbursements totaled $15,600 or 0.3% of commodity costs. Implementation of two strategies, integration of malaria commodities data into DHIS2 and facilitated supervision by district pharmacists, dramatically improved the GF core performance indicator for reporting of number of people treated appropriately for malaria. By early 2013, Kenya was rated "adequate" (i.e., B1 rating) for overall grant performance, which will ensure continued GF funding. Funding for facilitated supervision should be included in GF plans.

552

SEVERE NEUTROPENIA IN DENGUE: PREVALENCE AND SIGNIFICANCE

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Prolonged severe neutropenia unrelated to dengue is known to be associated with a higher risk for secondary infections. Peripheral neutropenia is commonly reported in dengue. However, its clinical significance is uncertain. We set out to determine the prevalence and duration of severe neutropenia in adult patients with dengue, and to investigate factors associated with severe neutropenia and its predictive capability for nosocomial infections or more severe clinical outcomes. We did a retrospective analysis of 1921 adult patients admitted to the Communicable Disease Center in Singapore between 2005 and 2008 with PCR confirmed dengue Severe neutropenia was defined as absolute neutrophil count ≤0.5x109/L, and dengue hemorrhagic fever (DHF) was defined according to the WHO criteria. The Kruskal-Wallis test was used to assess significance of continuous variables and x2 or Fisher's exact test for categorical variables, and logistic regression to identify independent factors associated with severe neutropenia. Our results showed that the prevalence of severe neutropenia is 11.8% (227/1921) with the lowest

neutrophil counts recorded on day 5 of illness. The median duration was 1 day. On the day of admission, only 2.4% had severe neutropenia. Age, Chinese ethnicity, inter-menstrual bleeding and hematocrit percentage were significantly associated with severe neutropenia in multivariate analysis. Severe neutropenia was not predictive for DHF, prolonged hospitalization stay or mortality. Our analyses also showed that severe neutropenia was not associated with a higher risk of secondary bacterial infections (pneumonia, urinary tract infection and *bacteremia*). In conclusion, severe neutropenia in adult dengue patients is frequent, but short-lived and not associated with nosocomial infections, prolonged hospitalization or increased mortality or DHF.

553

POTENTIAL HARM OF PROPHYLACTIC PLATELET TRANSFUSION IN ADULT DENGUE

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Transient thrombocytopenia is common in dengue. Concern of bleeding risk from severe thrombocytopenia may lead to preventive platelet transfusion. Data from small series in neonatal dengue shock syndrome and adult dengue fever did not show benefit. We studied all hospitalized adult dengue patients at Communicable Disease Centre, Singapore from 2005 to 2008 with a positive dengue polymerase chain reaction or serology (fulfilled World Health Organization 1997/2009 probable dengue criteria) whose platelet was lower than 20x10^9/L without bleeding. We aim to study potential benefits and harms of preventive platelet transfusion. Of 6234 hospitalized patients, 809 developed platelet count ≤20x10^9/L without bleeding, and 498 were transfused platelet. At baseline, transfused patients had more leukopenia (3.4 vs. 3.9, x10^9/L), lymphopenia (28% vs. 31%), thrombocytopenia (14 vs. 16, x10^9/L), and neutrophilia (52% vs. 46%), and higher AST (208 vs. 153, U/L) and ALT (117 vs. 84, U/L) levels ($p<0.05$). The two groups were similar in age, fever duration, systolic blood pressure and serum hematocrit. While transfused patients had higher platelet increment the next day (8 vs. 5, x10^9/L), they had more mucosal bleeding (18% vs. 9%), longer time to platelet ≥50x10^9/L (3 vs. 2 days) and hospital stay (6 vs. 5 days), and more severe dengue (20% vs. 15%) ($p<0.05$). There was no difference in all clinical (23% vs. 18%) and severe bleeding (3.4% vs. 1.3%), dengue hemorrhagic fever (17% vs. 15%), intensive care admission (3.4% vs. 1.3%) or death (0.2% vs. 0%). Preventive platelet transfusion was associated with higher platelet increment but appeared to cause potential harm in prolonging platelet recovery and hospital stay without reducing all clinical or severe bleeding.

554

EFFECTIVENESS OF ROTAVIRUS VACCINATION AGAINST SEVERE CHILDHOOD DIARRHEA - GUATEMALA, 2012

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Concerns remain about lower effectiveness and waning immunity of oral rotavirus vaccines in poor populations, where enteric co-infections, co-morbidities, malnutrition, and unusual rotavirus strains are common. We evaluated vaccine effectiveness against severe rotavirus disease in Guatemala, one of the first low-income countries to implement routine rotavirus vaccination in 2010. A case-control evaluation was conducted in inpatient and emergency department settings in 4 hospitals during 2012. Card-confirmed vaccine history was compared between case-patients (children with laboratory-confirmed severe rotavirus diarrhea) and 2 sets of controls: non-diarrhea hospital controls (matched by date of birth ±30 days) and rotavirus-negative diarrhea controls (adjusted for birth quarter). Vaccine effectiveness ((1-odds ratio of vaccination) x100%) was computed using logistic regression models. We enrolled 191 case-patients, 540 non-diarrhea controls, and 291 rotavirus-negative controls. Case-patients and controls were similar for breastfeeding, birthweight, maternal education, and socioeconomic variables. An uncommon G12P[8] strain, heterotypic to the vaccine strain, was identified in 90% of rotavirus cases. Effectiveness of a full vaccine series against severe rotavirus diarrhea was 66% (95% confidence interval [CI]: 37%-82%) with non-diarrhea controls, and 68% (CI: 41%-83%) with rotavirus-negative controls; partial vaccination (one dose) was 52% (CI: -85%-88%) and 61% (CI: 4%-84%) effective, respectively. No significant differences in effectiveness were observed between infants 6-11 months (83%; CI: 33-96) compared to children ≥12 months of age (64%; CI: 23-83) (P=0.3). Rotavirus vaccination provides protection through 2 years of life against severe rotavirus diarrhea caused by a heterotypic strain among Guatemalan children. This supports broader implementation of rotavirus vaccination in low-income countries where >90% of the half million annual global deaths from rotavirus occur.

555

DISPARITIES EXIST IN THE PROVISION OF TRAVELERS' DIARRHEA SELF-TREATMENT TO CHILDREN

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Travelers' Diarrhea (TD) is the most common illness in travelers, including children. The CDC Yellow Book endorses the use of antibiotics for self-treatment of traveler's diarrhea (STTD) in children but does not address the issue of anti-motility agents directly. This study is a retrospective review of antibiotic and anti-diarrheal prescriptions to military beneficiary children, ages 0-17, at pre-travel visits during 2010, traveling for <120days. Of 1557 visits, civilian medical providers accounted for 48%. Prescribing patterns were assessed and compared based on practice setting, provider specialty, and patient age. In total, 43% were not prescribed STTD. Across all ages, military clinics were more likely to prescribe STTD than civilian counterparts (OR=2.1, 95%CI 1.7, 2.6). This effect was seen in each age group and was most pronounced for infants and toddlers (OR=3.6, 95% CI 1.8, 7.2) and early adolescents (OR= 4.2, 95% CI 2.5, 7.0). Among military clinic visits, there was marked variation by specialty in the likelihood of STTD. Pediatric Clinics (PC) when compared toNon-Pediatric Primary Care Clinics (NPPCC) were more likely to provide STTD (OR=4.8, 95% CI 3.2, 7.2). This effect was seen in each age group, and most pronounced in school aged children (OR=7.1, 95% CI 3.2, 15.8) and infants and toddlers (OR=5.9, 95% CI 1.8-19.3). PC and Specialty Travel Clinics (STC) were similar in their use of STTD (OR=1.2, 95% CI 0.9, 1.7), except for infants and toddlers in which case STC were less likely to offer TD therapy (OR 0.36, 95% CI, 0.2-0.8). Loperamide use was low among both PC and NPPCC until middle adolescence when pediatric providers were more likely to prescribe it (OR 4.3, 95%CI 1.5, 12.1). STC were more likely to utilize loperamide than PC (OR 3.6, 95%CI 2.4, 5.3), particularly in school age (OR 2.4, 95%CI 1.1, 5.3) and middle adolescents (OR 2.6, 95% CI 1.0, 6.5). Pediatric travelers often do not receive CDC recommended care for STTD. Significant differences in STTD practice exist based on patient age and provider specialty and military affiliation. Evidence based guidelines for STTD specific to children, provider education, and decision support tools are needed.

556

INVASIVE *SALMONELLA* INFECTIONS IN AREAS OF HIGH AND LOW MALARIA TRANSMISSION INTENSITY IN TANZANIA

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Invasive salmonellosis is a major cause of childhood febrile illness and death across sub-Saharan Africa. The epidemiology of *Salmonella* Typhi and invasive nontyphoidal *Salmonella* (NTS) differs; *Salmonella* Typhi is often driven by environmental risk factors, while host-related factors such as HIV infection and malaria are associated with invasive NTS. We compared the prevalence of malaria and *bacteremia*, in particular invasive salmonellosis, among hospitalized febrile children aged 2 months to 13 years at 2 sites in Tanzania. Teule Hospital (TH) and Kilimanjaro Christian Medical Centre (KCMC) are located in areas of high and low malaria transmission intensity, respectively. Sites employed similar study protocols and participants were enrolled at TH from June 2006 through May 2007 and at KCMC from September 2007 through August 2008. Blood culture using BacT/ALERT, malaria microscopy with Giemsa-stained blood films, and HIV testing were performed. At TH, 3,639 children were enrolled compared to 467 at KCMC. Smear positive malaria was detected in 2,195 (60.3%) of 3,639 at TH and 11 (2.4%) of 460 at KCMC (p<0.001). Bacteremia was present in 336 (9.2%) of 3,639 at TH and 20 (4.3%) of 463 at KCMC (p<0.001). NTS was isolated in 162 (4.5%) of 3,639 children at TH and 1 (0.2%) of 463 at KCMC (p<0.001). *Salmonella* Typhi was isolated from 11 (0.3%) patients at TH and 6 (1.3%) at KCMC (p=0.008). With NTS excluded, the prevalence of *bacteremia* at TH was 5.0% and at KCMC 4.1% (p=0.391). HIV prevalence among enrollees at TH was 3.9% compared to 13.2% at KCMC (p<0.001). Where malaria transmission was intense, invasive NTS was common and *Salmonella* Typhi was uncommon, with the converse true where malaria transmission intensity was low. Bacteremia was more prevalent at TH than KCMC, but when NTS was excluded, there was no difference in proportions of bacteremic children between the sites. Invasive NTS and *Salmonella* Typhi may compete in as yet undetermined ways, and the interactions between these pathogens, the environment, and the host is a compelling area for future research.

557

BACTEREMIA IN CHILDREN UNDER FIVE YEARS ADMITTED WITH NON-MALARIA FEBRILE ILLNESS AT JINJA CHILDREN'S HOSPITAL, UGANDA

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Acute febrile illnesses are the leading cause of hospital admissions among children under five years of age in Africa. Malaria, viral and invasive bacterial infections are the most common causes. Making a definitive diagnosis is challenging in resource limited settings, and most acute febrile illnesses are managed presumptively as malaria with often serious consequences. The objective of the study was to determine the prevalence, clinical features and spectrum of bacterial aetiologies of *bacteremia* among children under 5 years of age admitted to Jinja Children's Hospital with antimalarial treatment despite negative malaria slide. A total of 250 children under 5 years admitted with acute febrile illness, receiving antimalarial treatment despite a negative malaria smear were enrolled into the study. Clinical assessment was performed and blood collected for culture and complete blood count performed at the Makerere University Medical Microbiology Laboratory. A total of 15 blood samples (6%) were contaminated and excluded in the final analysis. Bacteria were present in 44/235 samples giving a prevalence of 18.7%. *Staphylococcus aureus* was the commonest isolate 41% (18/44) followed by non typhoidal *salmonella* 25% (11/44), pseudomonas aeruginosa 11% (5/44) and streptococcus pneumonia 9% (4/44). The common clinical features among children with *bacteremia* were temperature ≥ 37.5^{0}C (86%), cough (84%), vomiting (47%), weight loss during the illness (45%), diarrhea (44%) and history of fever for ≥ 2weeks (30%). Bacteremia was significantly associated with fever lasting 2weeks or more (OR = 1.53; 95% CI, 1.05-2.24), history of weight loss during the illness (OR = 2.70; 95% CI, 1.37-5.34) and total White Blood Count Cell (WBC) ≥ 15,000cells/ul (OR = 2.02; 95% CI, 1.04-3.92). History of weight loss during the illness was an independent predictor of *bacteremia* (OR = 2.5; 95% CI, 1.26-5.00). Clinicians should have a high index of suspicion for *bacteremia* in children under 5 years with negative malaria slide especially those with history of weight loss. The prevalence of *bacteremia* in children admitted with acute febrile illness was high. Commonest bacterial pathogens were staphylococcus aureus followed by non typhoidal *salmonella*. Weight loss during the illness was an independent predicator of *bacteremia*.

558

FIRST YEAR FINDINGS FROM AN ACUTE FEBRILE ILLNESS SURVEILLANCE STUDY IN PUERTO RICO

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Dengue is endemic in Puerto Rico but little is known about its epidemiology in relation to other acute febrile illnesses (AFI). To study this, enhanced AFI surveillance was implemented at a large referral hospital in Ponce, Puerto Rico. Outpatients with fever or history of fever for ≤7 days were enrolled with informed consent and followed through their illness. Specimens including serum and nasopharyngeal swabs were collected and tested by PCR and immunodiagnostic methods as appropriate for *Leptospira* spp (Lepto), *Burkholderia pseudomallei (Burk)*, 5 enteroviruses (Entero), influenza A (Influ A), influenza B (Influ B), 12 other respiratory viruses including adenovirus, respiratory syncytial virus, metapneumovirus, and parainfluenza viruses 1-4, and 4 dengue viruses (DENV). 1,739 of 6,495 AFI patients seeking care were enrolled between May 2012-March 2013; 31.9% were hospitalized, 46.9% were female, and the median age was 12.7 years (range: 0-93 years). Pathogens were detected in 1,206 (69.4%) cases; 1 (0.1%) Lepto, 3 (0.2%) Burk, 39 (2.2%) Entero, 135 (7.7%) Influ A, 157 (9.0%) Influ B, 221 (12.7%), other respiratory viruses, and 651 (37.4%) DENV cases were identified. Almost all (99.0%) of the 417 DENV cases were DENV-1. Forty-two (2.4%) PCR positive co-infections were identified; 24 (57.1%) were DENV and a respiratory virus. Dengue patients were more likely to be admitted than other enrolled patients (OR 2.11, 95%CI 1.71-2.61) or influenza patients (OR 2.95, 95%CI 2.12-4.12); they were also slightly older than other patients (median age of 14.5 versus 9.9 years) but not influenza patients (14.5 versus 13.5 years). No pathogen-specific differences in gender were noted among infected patients. The majority of AFIs were caused by DENV, respiratory viruses,

and enteroviruses. Leptospirosis and melioidosis cases may be more focal and sporadic in nature requiring longer study in more than one site. There is some evidence that dengue cases may have more severe presentations when compared to other AFIs. Data for the complete first year will be analyzed and presented.

559

META-ANALYSIS OF URINE HEME DIPSTICK DIAGNOSIS OF *SCHISTOSOMA HAEMATOBIUM* INFECTION, INCLUDING LOW-PREVALENCE AND PREVIOUSLY-TREATED POPULATIONS

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Urogenital schistosomiasis remains endemic in many areas of sub-Saharan Africa. Current control is based on drug administration, targeted either to school-age children or to high-risk communities at-large. Urine dipsticks for detection of hematuria offer an inexpensive means for estimating infection prevalence. However, their diagnostic performance has not been extensively evaluated after community treatment or in areas with continuing low prevalence. The objective of the present study was a meta-analysis of dipstick accuracy for *Schistosoma haematobium* infection in endemic regions, with special attention to areas where infection intensity or prevalence is low. Studies were identified by search of online databases and hand search of existing study archives. Eligible studies included population surveys, irrespective of date, location, or language, that compared dipstick diagnosis of *S. haematobium* infection to standard egg-count parasitology. For 95 included surveys, variation in dipstick sensitivity and specificity was evaluated according to study size, age- and sex-specific participation, region, local prevalence, treatment status, and other factors potentially affecting test performance. Independent of prevalence, greater accuracy was seen in surveys of school-age children, whereas performance was less good among surveys performed in North Africa. By hierarchical summary ROC analysis, overall dipstick sensitivity and specificity for detection of egg-positive urines were estimated at 81% and 89%, respectively. Sensitivity was lower among treated populations (72%) and in population subgroups having lower intensity infection (65%). When the insensitivity of egg counting was considered, and diagnosis was instead inferred from combined hematuria + egg count findings, overall dipstick sensitivity/specificity was 82%/97%, but with significantly better sensitivity (92%) in higher prevalence settings. This analysis suggests that dipsticks will continue to serve as useful adjuncts for monitoring community prevalence following implementation of urogenital schistosomiasis control.

560

MICRO-GEOGRAPHICAL HETEROGENEITY IN *SCHISTOSOMA MANSONI* AND *S. HAEMATOBIUM* INFECTION AND MORBIDITY IN A CO-ENDEMIC COMMUNITY IN NORTHERN SENEGAL

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Schistosoma mansoni and *S. haematobium* are co-endemic in many areas in Africa. Yet, little is known about the micro-geographical distribution of these two infections or associated disease within such foci. Such knowledge could give important insights into the drivers of infection and disease and as such better tailor schistosomiasis control and elimination efforts. In a co-endemic farming community in northern Senegal (n=599), we studied the spatial distribution of *S. mansoni* and *S. haematobium* single and mixed infections (by microscopy), *S. mansoni*-specific hepatic fibrosis, *S. haematobium*-specific urinary tract morbidity (by ultrasound) and water contact behavior (by questionnaire). The Kulldorff's scan statistic was used to detect spatial clusters of infection and morbidity, adjusted for the spatial distribution of gender and age. *S. mansoni* and *S. haematobium* infection densities clustered in different sections of the community (p=0.002 and p=0.023, respectively). This divergent pattern was related to the use of different water contact sites. Furthermore, the *S. mansoni* infection cluster overlapped with that of severe hepatic fibrosis. Within that cluster, more severe hepatic fibrosis clustered in a small group of adults living adjacent to the most frequently used water contact site (RR=6.3; p=0.043). *Schistosoma* infection and associated disease showed important micro-geographical heterogeneities with divergent patterns for *S. mansoni* and *S. haematobium* in this Wolof community. Further in depth investigations are needed to confirm the micro-geographical segregation of *S. mansoni* and *S. haematobium* infection and the strong geospatial clustering of chronic disease in other settings and over time, and to explain these phenomena. Yet, the present study indicates that micro-geographical patterns should not be overlooked in schistosomiasis-related research and control, and are crucial for elimination efforts.

561

HIGH *SCHISTOSOMA MANSONI* DISEASE BURDEN IN A RURAL DISTRICT OF ZAMBIA

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Schistosoma mansoni is endemic in most parts of rural Zambia, and complications such as hepatosplenomegaly, ascites and portal hypertension are commonly reported. We conducted a cross-sectional survey to determine the burden of *S. mansoni* disease and associated risk factors among 754 people (age range 7-50 years; mean 28.3) in four rural communities (Luampa, Mangango, Mwadasengo and Namando) of Kaoma district between September and October 2012. Kato-Katz technique was employed using duplicate stool smears to detect *S. mansoni* eggs. Intensity of infection was determined by obtaining average egg counts from two readings. Hepatosplenic disease was assessed using physical examinations and ultrasonography. The overall prevalence of *S. mansoni* infection and geometric mean egg count (GMEC) was 42.4% (304/717) and 86.6 epg (95% C.I 75.6, 99.6), respectively. Heterogeneity in disease distribution was observed within and among communities with various ecological patterns. Infection rates in Namando were 10 times more compared to Mangango (95% CI 5.20, 19.82; p<0.001) while Mwadasengo had high GMEC. High GMEC (119.2epg; 95% CI 78.9, 178) were observed in the age group 11- 14 years followed by the age group 15 - 18 years (113.4; 95% CI 79.0, 163). Although females had high infection rates than males (45.0% vs. 38.0%; p. value=0.069), higher GMEC (209.5epg vs. 191.2epg; p. value= 0.655) were recorded in males. *S. mansoni* /hookworm and *S. mansoni* /malaria co-infections were detected in 12.3% and 5.2% of the population, respectively while there was absolutely no *S. haematobium* detected.Ultrasonography detected hepatosplenic disease in 28 % (199) of the 710 participants examined, with the majority (87%) detected in individuals above 15 years of age. Severe hepatosplenic disease was detected in 42(6%) of the participants. The findings highlight high burden of *S. mansoni* disease in this area and calls for immediate interventions to avert complications associated with the disease.

562

IMPACT OF TWO ROUNDS OF MASS DRUG ADMINISTRATION FOR SCHISTOSOMIASIS CONTROL IN WESTERN KENYA: COMPARISON BETWEEN COMMUNITY WIDE TREATMENT AND SCHOOL-BASED TREATMENT IN HIGH PREVALENCE AREAS - THE SCORE PROJECT

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There has been increased global commitment for schistosomiasis control through mass drug administration programs through either school based or community wide treatments. As part of the ongoing Schistosomiasis Consortium for Operational Research and Eliminaiton (SCORE) projects in western Kenya, we evaluated the effect of two rounds of Praziquantel on schistosomiasis prevalence and egg intensity and compared community wide treatment (CWT) where the whole community was treated, to school based treatment (SBT) where school age children were treated by health teachers in schools. Among 150 communities participating in the SCORE project in areas with >25% prevalence in western Kenya, data from 33 communities belonging to either CWT or SBT arms that have been surveyed three times so far is presented. Written informed consent and assent were obtained from parents/ legal guardians and minors respectively. Three consecutive stool samples were collected from 100 children aged 9-12 years in each school and two slides prepared from each stool. The Kato/Katz method was used to identify *Schistosoma mansoni, Trichuris trichura*, and *Ascaris* eggs in the fecal material. Data was analyzed using SPSS software and group means compared using ANOVA. The overall prevalence and egg intensity (eggs per gram, epg) in CWT before MDA followed by two rounds of MDA of *S. mansoni* were 56.8% (Range: 20-100), 127.41 epg; 45.4 % (Range: 14.3-90.3) 74.40 epg and 35.3% (Range: 8.6-84.8) 49.4 epg respectively. There was significant reduction in prevalence levels (P= 0.022) with no significant difference in egg intensity (P=0.99). In the SBT arm, the prevalence and egg density pre and post two rounds of MDA were 60.92% (Range: 59.66-62.17), 126.64 epg; 51.33% (Range: 50.8-53.4), 126.63 epg and 38.8% (Range: 10.2-83.7), 53.3 epg respectively. There was significant reduction in both prevalence and egg density after two rounds of MDA (P<0.01). Following two rounds of mass drug administration; there was significant effect in both prevalence and egg density in SBT while in CWT arm, the effect was only significant in the prevalence.

563

CHALLENGES IN IMPLEMENTING COMMUNITY WIDE TREATMENT FOR THE CONTROL OF SCHISTOSOMIASIS IN WESTERN KENYA - THE SCORE PROJECT

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In areas with high prevalence for schistosomiasis, WHO recommends community-wide treatment (CWT) be provided rather than school-based treatment that is used in lower prevalence areas. We conducted CWT in 75 villages in western Kenya employing community health workers (CHW) as drug distributors. We compared CHW reported coverage with an independent household coverage survey to identify factors that may influence CHW reporting. Simple stratified random sampling was used to select 15 households in each village. A structured questionnaire was used to determine treatment coverage levels as well as levels of drug side effects experienced. A total 1125 households in 75 villages were visited. Up to 63.9% (37.8-94.1%) reported having been treated compared to the CHWs reported coverage of 86% (85.1-106.7%) in the same villages (P<0.0001). Only 20.6% reported being absent during the treatment with 51.7% reporting that the CHW did not visit their homes to offer treatment. Few people declined treatment for fear of side effects (0.65%). About 2.9% of the population surveyed reported having not heard about the project. Only 0.32% felt they were not sick hence didn't need drugs, while 0.55% reported they were pregnant and 0.08% were influenced by rumors. Up to 20.3% were children under 5 years. Of the total population surveyed, only 25.9% experienced side effects with abdominal pain being the most frequent complaint (46.0%), followed by diarrhea (31.7%). The significant difference between CHW-reported coverage and house-hold survey coverage exposed the challenges faced in the CWT strategy which include logistical problems in ensuring prompt assessment to allow for follow-up treatment where needed, early identification of village-specific factors that may influence coverage levels, management of community fears related to drug side effects and strategies for ensuring maximum coverage of school-age children at the community level.

564

HIGH PREVALENCE OF *SCHISTOSOMA JAPONICUM* IN HUMANS AND BOVINES FROM NORTHERN SAMAR, THE PHILIPPINES

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Schistosoma japonicum is the causative agent of schistosomiasis in the Philippines, China and parts of Indonesia. In the Philippines, 6.7 million people live in endemic areas with 1.8 million having direct exposure through daily water contact activities. As a zoonosis *S. japonicum* infects over 40 mammalian species, including water buffalo which have been shown to be major reservoir hosts in China. In the Philippines, water buffalo (Carabao) have been considered unimportant hosts due to low prevalence and infection intensity found in previous studies. Six barangays from the Northern Samar municipality of Palapag were surveyed to determine the role of carabao and cattle in the Philippines Bovine samples were examined with an improved microscopy technique (formalin-ethyl acetate sedimentation (FEA-SD) and qPCR analysis, while human samples were examined by KK (Kato-Katz) in addition to qPCR. High *S. japonicum* prevalence was found in humans when using qPCR (90.36%), while KK showed a much lower prevalence (22.86%). High prevalence was also found in bovines when using FEA-SD (72.00%) and qPCR (81.50%). Intensity of infection varied for bovines with cattle having a higher epg (eggs per gram) (2.23) than carabao (1.49). The Bovine Contamination Index was calculated using the combined carabao and cattle epg (1.74) and showed that each bovine was excreting an average of 42,750 eggs into the environment daily. Bovines also had a high prevalence of *Fasciola gigantica* infection by both FEA-SD (95.00%) and qPCR (95.65%) techniques. Bovines, particularly carabao, due to their high rate of daily water contact, likely play a more substantial role in schistosomiasis transmission in the Philippines than has been reported previously.

565

SPATIAL PREDICTION OF HUMAN *SCHISTOSOMA JAPONICUM* INFECTION IN THE PHILIPPINES: TOOLS TO SUPPORT DISEASE ELIMINATION

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Schistosoma japonicum infection is endemic in 28 of the 80 provinces of the Philippines and the most recent data on schistosomiasis prevalence has shown considerable variability within provinces. In order to increase the efficient allocation of parasitic disease control resources in the country, we aimed to describe the spatial variation in *S. japonicum* risk across the Philippines, quantify the role of the physical environment in driving the spatial variation of *S. japonicum* and develop a predictive risk map of *S. japonicum* infection. Data on *S. japonicum* infection from 35,754 individuals across the country were geolocated at the barangay-level and included in the analysis. The analysis was then stratified geographically for Luzon, the Visayas and Mindanao. Non-spatial multivariable models of *S. japonicum* prevalence were built, including age and sex of individuals and environmental variables (rainfall, land surface temperature and distance to inland water bodies) as predictors; residual spatial dependence in *S. japonicum* prevalence was investigated using semivariograms. Zero-inflated binomial (ZIB) Bayesian geostatistical models of *S. japonicum* prevalence were developed, and diagnostic uncertainty was incorporated.Results of the analysis show that in the three regions, males and individuals aged≥20 years had significantly higher prevalence of *S. japonicum* compared to females and children <5 years. The role of the variables of physical environment was different between regions of the Philippines. The geographical distribution of *S. japonicum* risk was widespread in the Visayas whereas in Luzon and Mindanao it was much more focal. This analysis reveals significant spatial variation in *S. japonicum* infection risk in the Philippines. This suggests that a spatially targeted approach to schistosomiasis interventions, including mass drug administration, is warranted. When financially possible, additional schistosomiasis surveys should be prioritized to high risk areas identified by our study in Luzon which are currently underrepresented in our database.

566

IDENTIFICATION AND CLONING OF *TRIATOMA DIMIDIATA* IMMUNOGENIC SALIVARY PROTEINS TO DEVELOP TRIATOMINE EXPOSURE IMMUNOASSAYS

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Triatoma dimidiata is a vector of Chagas disease in sourthern Mexico. Current methods to assess triatomine infestation usually lack sensitivity and precision and also are time consuming and costly. New methodologies are required to monitor bug populations in endemic regions of Chagas disease. Saliva of hematophagous bugs contains proteins that can elicit an antibody response. This has been used as an epidemiological tool and biological marker of exposure to disease vectors. We used sera from four Balb/c mice exposed to *T. dimidiata* bites to evaluate antibodies against salivary proteins by Western Blot. Immunogenic salivary proteins of ~14, ~18 and ~79 kDa were recognized by all mouse sera. The 79 kDa protein may be an apyrase, which has cross reactivity with other arthropods such as *Aedes aegypti*. Besides, 14 and 18 kDa salivary proteins may be specific of triatomines and represent good candidates for molecular markers of exposure to *T. dimidiata* saliva. Therefore, based in sequences previously reported in Gene Bank we designed primers to amplify 14.6 and 18 kDa *T. dimidiata* proteins. Then we isolated mRNA from salivary gland of *T. dimidiata* bugs and we obtained the amplicons by RT-PCR. They were cloned in a pGex plasmid using a double digestion strategy with BamI and XhoI enzymes. Both plasmid will be expressed in a procariot system to obtaining recombinant proteins, those will be used to develop anti-*T. dimidiata* salivary proteins immunoassays to monitor vector exposure.

567

GEOGRAPHIC DISTRIBUTION OF *TRIATOMA DIMIDIATA* (*REDUVIIDAE: TRIATOMINAE*) IN NORTHERN BELIZE

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The triatomine vectors responsible for transmission of *Trypanosoma cruzi*, the causative agent of Chagas disease, are widespread throughout much of Central and South America. As the influence of this neglected disease is continuously augmented by globalization, an understanding of disease epidemiology must include documentation of vector distribution across Chagas endemic regions. *Triatoma dimidiata* is the sole Chagas vector reported from Belize, yet literature pertaining to the local ecology and control of this vector has been scarce since initial reports from the 1960s. A recent study provided valuable information on vector population dynamics and distribution in southern Belize, yet vector distribution and infection rates in northern Belize remain unknown. Here, an initial report regarding vector distribution throughout northern Belize and localized infection rates of vector populations is provided. A brief comparison of collection methodologies is described and early recommendations for regional vector control and surveillance are presented.

568

THE VECTOR MOSQUITO *AEDES AEGYPTI* AT THE MARGINS: SENSITIVITY OF A COUPLED NATURAL AND HUMAN SYSTEM TO CLIMATE CHANGE

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Dengue viruses circulate between mosquito vectors and humans, causing nearly 400-million dengue infections annually. In the last decade, the Americas have experienced a dramatic increase in severe disease cases (dengue hemorrhagic fever), with devastating public health consequences. Of particular concern is the potential for the expansion of intense dengue virus transmission into cooler, high altitude cities that are presently outside of transmission zones but may be at risk under scenarios of climate change, such as Mexico City. To address this problem we are employing a coupled natural and human systems approach to explore the ecology of *Aedes aegypti*, the mosquito vector of dengue viruses, in Mexico. A field study is being conducted along a transect from Veracruz City to Puebla City, ranging from relatively warm and wet low-elevation coastal environments with well established vector mosquito populations and intense dengue virus transmission, to comparatively cool and dry high-elevation mountainous areas which currently are free of the mosquito vector and local virus transmission. Along the transect we are measuring how climatic, socio-economic and infrastructure factors are coupled with *Ae. aegypti* abundance. These data are being synthesized into spatially and temporally predictive models to examine if, how, and why the range of the dengue vector *Ae. aegypti* may change in the future. Observational and modeling results from two field seasons will be presented, indicating strong linkages between climate and mosquito presence and abundance.

569

INVESTIGATING VARIABILITY IN THE GUT MICROBIOTA OF THE DENGUE VECTOR *AEDES AEGYPTI*

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The *Aedes aegypti* mosquito gut microbiome has the capacity to dramatically alter the success of dengue virus infection in the mosquito midgut. Moreover, it has been shown that certain species of bacteria are much more effective at preventing the mosquito from becoming infected with the virus. Manipulation of the mosquito gut microbiota is therefore one potential approach to reduce disease transmission. In order to better inform control strategies that involve the mosquito microbiome, we are investigating factors that determine its size and composition. In the present work, we reared multiple field and laboratory strains of *Ae. aegypti* in a controlled laboratory environment. The field strains were collected from Singapore, Thailand and St. Kitts and have been raised in the laboratory for 6-8 generations since collection of the parental generation. The laboratory strains we used were Rockefeller (origin: Caribbean, ~1930s), Orlando (origin: Orlando, FL, ~1940s) and Waco (origin: Waco, TX, ~1987). To standardize exposure to environmental microbes, we mixed larval water between strains multiple times during larval and pupal development. We then assessed variability in the number and species distribution of culturable microbes in the midguts of sugar and bloodfed females from each strain. We found substantial variation between strains in the size of the midgut microbiome in sugar fed individuals and in response to bloodfeeding. Investigations into the molecular and genetic basis of strain-specific differences in the culturable gut microbiome and the impact on dengue virus infection will be discussed.

570

TOWARD AN UNDERSTANDING OF HOST PREFERENCE IN *TRIATOMA SANGUISUGA* (*HEMIPTERA:REDUVIIDAE*)

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Although rare in occurrence, autochthonous transmission of *Trypanosoma cruzi*, the etiologic agent of Chagas disease, within the United States is an area of growing concern. The arthropod vector that has been most implicated in these cases is the Eastern Bloodsucking Conenose (*Triatoma sanguisuga*). In southeastern Louisiana, the prevalence of *T. cruzi* in the adult population of *T. sanguisuga* has been found to be near 60%. Given this high prevalence, it seems likely that ecological and behavioral characteristics of the vector prevent frequent transmission of the parasite to humans. However, little is known with respect to the natural ecology of *T. sanguisuga*, including whether it is closely associated with a specific mammalian host as has been found for other closely related *Triatoma* species in the southwestern United States. We undertook a multi-year, ecological study of the species to better understand its preferred blood meal sources. Because sylvatic rodents have been found to be the principal host for other *Triatoma* species, in order to test them for *T. cruzi,* a sample of rodents was taken from a known *T. sanguisuga* habitat. Over three years, a total of 59 rodents were collected from the same parcel of land and tested for infection by PCR for *T.cruzi* kDNA. To complement these collections, late instar and adult *T. sanguisuga* specimens were also collected from this area and are being analyzed by PCR for both *T.cruzi* infection and blood meal source. This analysis of > 500 specimens is underway and will be presented at the meeting. Preliminary results from an initial sample of thirty rodents suggest a relatively high prevalence (60%) of *T.*cruzi infection. The three species of rodents contained in this sample tested positive for *T. cruzi* at varying levels: *Neotoma floridana* (67%), *Peromyscus gossypinus* (58%), and *Mus musculus* (0%). These data will allow us to better understand the host feeding preferences of *T. sanguisuga* and therefore gain a more complete understanding of the sylvatic transmission cycle of *T. cruzi* in the southcentral United States.

571

QUANTIFYING IMPACT OF MOSQUITOES ON QUALITY OF LIFE

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New Jersey, like many eastern states, has a persistent problem with the Asian tiger mosquito. This species and other mosquitoes reduce residents' quality of life through discomfort and possible risk of disease. To guide a comprehensive area-wide pest management project to control *Aedes* albopictus in two counties in New Jersey, we quantified the impact of mosquitoes on residents' quality of life. We interviewed residents of 121 randomly selected households in both counties between October and November 2010. We asked residents about their experience with mosquitoes in their neighborhood, the importance of mosquito control compared to other public services (1=not important, 5=extremely important), and rated residents' utility based on paired comparisons to known health states on the EuroQol scale from 0 (death) to 1 (perfect health). The majority (54.6%) of respondents considered mosquitoes to be a problem, rating its severity as moderate (30.6%), severe (12.4%), or extremely horrible (11.6%). Respondents reported an average (±SD) of 7.1±4.0 mosquito bites in a typical summer week. Mosquitoes prevented 59.5% of residents from enjoying their outdoor activities at least to some extent. Residents rated their mosquito experience during that summer on a scale of 100 (no mosquitoes) to 0 (mosquitoes invasion) at 56.7±28.7, and their overall utility at 0.87±0.03 comparable to being moderately anxious or depressed. Respondents rated the importance of enjoying porch and yard outdoors activities without mosquitoes (4.7±0.8) equal to that of neighborhood safety and higher than that of a clean neighborhood (4.6±0.9). In conclusion, these New Jersey residents report a 0.13 decrement in utility due to mosquitoes, possibly comparable to the decrement associated with depression, and rate mosquito control as an extremely important element of public service.

572

GENETIC PEST MANAGEMENT AND SOCIETY: AN INTERDISCIPLINARY ASSESSMENT OF CURRENT AND EMERGING TECHNOLOGIES FOR DENGUE CONTROL

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Dengue fever has been receiving increased attention from scientists and health professionals as a neglected tropical disease escalating in prevalence throughout the globe. Given the complex nature of the dengue virus and its primary mosquito vector, *Aedes aegypti*, several methods involving genetically engineered mosquitoes are being researched as potential solutions to issues of dengue control. However, controversies surrounding the implementation of biotechnologies in the past suggest the need to pay greater attention to the social and cultural contexts in which these technologies interact. We bring an interdisciplinary perspective to an assessment of current and emerging pest management technologies for dengue, with particular attention to the broader social, cultural, economic,

and ecological settings wherein these technologies may be used. Based on our assessment, controlling the dengue virus and its primary vector will require a multifaceted approach. Control programs need to be assessed individually according to their specific context in order to be successful and sustainable. We survey multiple databases covering the fields of biology, communications, entomology, ecology, epidemiology, economics, policy, and genetics. To assist policy makers in deciding how best to ease the burden of dengue, we develop a decision tree that suggests actionable solutions to some of the complex and multifaceted issues of dengue control. Scientists, policymakers, non-government organizations, and the public are currently and will be involved in complex decisions with constantly changing variables. It is our hope that incorporating a contextually specific and interdisciplinary approach to dengue control programs will enhance the research being conducted and help inform policy decisions, thereby improving future outcomes for all involved in the process and all those affected by dengue each year.

573

STATE-WIDE SCREENING OF *AMBLYOMMA AMERICANUM* FOR *EHRLICHIAE* AND SPOTTED FEVER GROUP *RICKETTSIAE* SPECIES IN VIRGINIA USING A NOVEL MULTIPLEX REAL-TIME PCR ASSAY

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The population of the lone star tick, *Amblyomma americanum*, has expanded in North America over the last several decades owing to expanding deer populations. *A. americanum* is known to be an aggressive and non-discriminatory biter and is by far the most common human-biting tick in Virginia. It is also a known carrier for several bacterial diseases, making it an increasingly important disease vector. Few studies of human pathogen prevalence in ticks have been conducted in the state of Virginia since the mid-twentieth century. With renewed interest in tick surveillance in Virginia, we undertook this study to survey *A. americanum* populations from around the state for the presence of three *Ehrlichia* species (*E. chaffeensis, E. ewingii,* and Panola Mountain *Ehrlichia*), and three spotted fever group *Rickettsiae* (SFGR) (*R. amblyommii*, *R. parkeri,* and *R. rickettsii*) using a novel six-plex real-time PCR assay. Our studies revealed a high prevalence (50-80%) of *R. amblyommii,* a non-pathogenic SFGR in all areas surveyed, along with a presence of all three *Ehrlichia* species (1-22%). *R. parkeri*, previously only known to be harbored within Virginia's *Amblyomma maculatum* ticks, was found in *A. americanum* in several surveyed areas within two regions with established *A. maculatum* populations. This suggests that within those two geographic regions, *A. americanum* and *A. maculatum* share one or more reservoir hosts. *R. rickettsii* was not found in any sample tested. Our study provides the first state-wide screening of *A. americanum* ticks in recent history and indicates that exposure to *R. amblyommii*, and to *Ehrlichia*e may be common. The high rate of *R. amblyommii* suggests serology may be misleading in clinical cases of tick-borne disease, and that *Ehrlichia* suspicion should be increased. These data may be of relevance to other regions where *A. americanum* is prevalent.

574

CHARACTERIZATION OF RELATIONSHIPS AND DEMOGRAPHIC PARAMETERS OF FOUR *ANOPHELES PUNCTULATUS* SIBLING SPECIES OF PAPUA NEW GUINEA BY GENOME SEQUENCING

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Members of the *Anopheles punctulatus* (AP) group are the principal vectors of malaria and lymphatic filariasis in the Southwest Pacific. This group is comprised of 13 sibling species, five of which are considered to be major vectors in Papua New Guinea. Understanding species relationships and population diversity has important implications for the implementation of vector control programs. Unfortunately, very limited genetic data is available for AP mosquitoes, in part due to their evolutionary distance to *An. gambiae* and to the extensive divergence among AP sibling species, as reported previously. Here, we sequenced the genomes of four AP sibling species by shotgun sequencing and generated 74 to 340 million reads for each species. We *de novo* assembled each species' genome independently generating an average of 26,163 contigs (range: 14,407 – 41,925) with an average assembly size of ~151 Mb (146 - 161) and an N50 of 9,980 (4,664-16,229). We aligned the contigs from each genome to produce a total of 82,651,073 nucleotides aligned in all four species (representing ~30% of the genome) and sequenced at greater than 10 X coverage in each species. Using these aligned sequences and highly redundant next generation sequencing data from each species, we were able to identify several million fixed differences (positions variable between species) and DNA polymorphisms (variable within a species). This data allows us to i) rigorously examine the phylogenetic relationships among these sibling species, ii) query the extent of introgression between species and iii) characterize the effective population size and population dynamics of each species.Our findings will provide a framework for better understanding how alleles could be shared within and between species. With improved knowledge of each species ecology and evolution, our study can contribute to improve integrated mosquito management strategies and improve malaria and lymphatic filariasis elimination efforts in this part of the world.

575

VECTORBASE COMMUNITY SUBMISSION SYSTEM

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VectorBase is a NIAID-funded Bioinformatic Resource Center that provides resources to investigate vectors of human pathogens. Currently we have the genomes of 11 vectors: *Anopheles gambiae, A. darlingi* and *A. stephensi* (malaria); *Aedes aegypti* (dengue and yellow fever); *Culex quinquefasciatus* (filariasis); *Rhodnius prolixus* and *Glossina morsitans* (trypanosomiasis); *Ixodes scapularis* (lyme disease); *Lutzomyia longipalpis* and *Phlebotomus papatasi* (leishmaniasis); and *Pediculus humanus* (louse-borne typhus, trench fever and louse-borne relapsing fever). In addition, for these and other vectors we have transcriptomes, proteomes, other "omics" data and, population data such as single-nucleotide polymorphisms (SNPs) and insecticide resistance phenotypes. In order to help scientists in the process of improvement or development of new strategies for controlling, or even eradicating vector borne diseases, as well as support basic science, VectorBase is committed to improving the system for community data submission, which has been divided in four sections as follows: 1. Gene annotation (models in GFF3 or fasta format) and metadata (gene name, symbol and description), which are meant to improve the organisms gene sets, through our Community Annotation Portal (CAP); 2. Gene transcript and protein data coming from colonized

or wild organisms, may be submitted for display on VectorBase using the expression browser and genome browser; 3. Linking genes to publications allows researchers to share their papers with the VectorBase community, making them visible on the VectorBase genome browser; 4. Phenotype data in the insecticide resistance database (IRbase), or variation data (*i.e.*, SNPs) in the population biology browser (PopBio). To submit, go to our community section at either our website home page or navigation tab, *www.vectorbase.org*. Attend this poster for an overview of the submission system, its ongoing developments, and discussion of suitability of this tool for the research community needs. Help or comments: *info@vectorbase.org*. Tutorials: *www.vectorbase.org/tutorials*.

576

MATERIAL SCIENCE AND PARATRANSGENESIS: AN UPDATED APPROACH TO CONTROL TRANSMISSION OF LEISHMANIASIS BY SAND FLIES

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Leishmaniasis continues to be considered one of the most neglected tropical diseases in the world. No vaccines are currently available, and the best methods for control involve the use of chemical pesticides that not only carry environmental toxicity risk but also promote resistance in vector populations. Paratransgenesis, often referred to as a "Trojan Horse" approach to vector control, is an alternate approach to reduce vector competence by genetically manipulating symbionts commonly found in disease vectors. These symbionts are then reintroduced to the vector for colonization of the midgut and production of effector molecules that negatively interfere with pathogen development. A paratransgenesic platform has been tentatively developed for sand fly control and possible reduction or elimination of *Leishmania* transmission in endemic areas. In this study, we have engineered two bacterial species, *Pantoea agglomerans* and *Bacillus subtilis*, to constitutively secrete an inactive antimicrobial peptide molecule. These bacteria were fed to 2nd instar larvae of *Phlebotomus papatasi* utilizing a novel bioencapsulation method and the emerging adults were monitored for colonization of their midgut. Paratransgenic sand flies were allowed to feed while gut colonization was continuously monitored. Flies that remained colonized were mated to assess fitness (e.g., survival and fecundity of females) and the possibility of vertical transmission. We are also assessing direct effect(s) of feeding melittin to adult sand flies. Our results suggest that bioencapsulation can be effectively used to deliver genetically modified bacteria to sand flies, and explores the effects of mellitin on sand fly physiology.

577

EMERGING PARASITE OR INCREASED AWARENESS? THE STORY OF *HYPODERMA TARANDI*

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The purpose of this report is raise awareness of clinical manifestations and diagnosis of myiasis caused by larvae of *Hypoderma tarandi*, a warble bot fly, widely distributed in the habitats of caribou and reindeer in Sub-Arctic regions of North America and Europe. We report the clinical history of 7 individuals including five children suffering from myiasis localized to the forehead and of additional three cases in which larvae was extracted from the eye globe, causing blindness in two patient. A diagnosis should be evoked in people seeking medical care in August -December due to recurrent migratory localized swellings of the forehead, enlargement of occipital lymph nodes with/without eye complaints. Such patients should be asked about recent trips (last summer-autumn) to Sub-Arctic regions. We wish to emphasize the importance of an integrated approach to diagnose this severe condition. Since the definitive confirmation by microscopy or genome sequencing requires extraction of a larva, meanwhile serological verification takes time to perform; we suggest that ivermectin should be given on clinical suspicion alone. We also wish to stress the importance of publishing case reports in the local medical press. Our first publication helped in the recognition of symptoms and in shortening diagnostic delay in quite a few patients including 14 children who were diagnosed in Northern Norway during autumn 2012. Diagnosed was confirmed serologically in all cases showing presence of antibodies against Hypodermin C, an enzyme indicative of *Hypoderma spp.* larva infestation.

578

HIGH PROPORTION OF HUMAN BLOOD MEALS IN *TRIATOMA SANGUISUGA* INFECTED WITH *TRYPANOSOMA CRUZI* SUGGESTS POSSIBLE EPIDEMIOLOGICAL THREAT OF CHAGAS DISEASE IN LOUISIANA

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Trypanosoma cruzi, the causative agent of Chagas disease, is transmitted by hematophagous insects called triatomines or "kissing bugs". In 2007, the first autochthonous case of vectorial transmission of Chagas disease in Louisiana (the sixth in the US) was described. 1 *Triatoma sanguisuga* was the species suspected to be responsible for this transmission, but little is known about its ecology and behavior. We report for the first time the predominant vertebrate blood meal sources of this species. A sample of 49 *T. sanguisuga* collected at the location of the first described case of vectorial transmission in Louisiana was used for the study. The infection of the bugs with *T. cruzi* was determined by PCR using TCZ primers. 2 To determine the blood sources of individual bugs, we used a recently described assay, amplifying 12S rDNA from the abdominal content of the bugs using vertebrate universal primers, followed by cloning and sequencing of the amplicons. 3 The *T. cruzi* infection prevalence of the bugs was 55%. Blood sources were successfully determined for 43 of the 49 bugs. On average, the number of blood source species detected was 1.6/bug; the highest was blood from four different species detected in a same bug. Surprisingly, the American green tree frog was the predominant blood source found. This species had never been described before as a blood source for any species of triatomine. Human was the second most detected blood source: 48.8% of the bugs had fed on human, and 10 different human 12S haplotypes were found, showing that at least 10 people had been fed on. The other blood sources were raccoon, cow, dog, squirrel, cat and Eastern woodrat. Almost 40% of the bugs which fed on human were infected, suggesting the potential of Chagas disease transmission to human in Louisiana.

579

ECOLOGY OF CUTANEOUS LEISHMANIASIS IN SINAI: LINKING PARASITES, VECTORS AND HOSTS

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Cutaneous leishmaniasis (CL) is a neglected clinical disease with high prevalence in northern and eastern parts of Egypt. Field investigations across the Sinai Peninsula from January 2005 to December 2011 revealed that only the zoonotic transmission cycle is widespread in this region, with 400-500 cases reported to clinics annually. CL is restricted to Northern Sinai districts along the northeastern border of Egypt. This study identified potential vectors and reservoirs involved in the regional transmission cycle. Three sand fly species (*Phlebotomus*) were detected: *P. papatasi*, *P. sergenti*, and *P. kazeruni*; of these, 0.41% of *P. papatasi* were found infected with *Leishmania*-like flagellates, but *P. sergenti* and *P.*

kazeruni showed no evidence of infection. In rodent populations, positive *Leishmania*-amastigote impression smears were recovered from *Gerbillus pyramidum* (14/31), *Gerbillus andersoni* (5/23), *Rattus rattus* (4/12). Although, this long-term study covered many districts of Sinai, only sand flies and rodents within El-Hassan, Rafah, and Beer Lehfen districts tested positive for infection. Restriction fragment length polymorphism revealed that only *L. major* was circulating in the area, with no evidence for the presence of *L. tropica*, except in five isolates from El Barth, Rafah, in 2005. Finally, an ecological niche modeling approach was used to test linkages between vector and pathogen species across the study region via analytical approaches and data streams that are completely independent of the field data.

580

DENGUE SURVEILLANCE SYSTEM IN ACTION IN THE PHILIPPINES

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Dengue is the most important mosquito-borne viral disease in the tropics particularly in the Philippines. This study aims to present the country's dengue surveillance system in action and the status of dengue surveillance data. The roles of the Epidemic Surveillance and Response (ESR) and the Philippine Integrated Disease Surveillance and Response (PIDSR) of the Department of Health will be discussed. ESR is the country's innovative way to participate in the World Health Organization's challenge to reduce morbidity and mortality of dengue. PIDSR aims to harmonize all existing disease surveillance systems to strengthen the capacity of the local government units (LGUs). Surveillance system involves the stepwise monitoring of the spectrum of dengue illness and disease reporting from the smallest administrative division called barangay health centers (or Rural Health Units, RHUs) to district or provincial hospitals, to regional hospitals, and then to the National Epidemiology Center by respective advocates, coordinators and officers at different hierarchical levels with PIDSR training. Reported national dengue cases in 2008-2010 comprised 90.08% (249,883) admitted in hospitals, 4.15% (11,522) not admitted, and 5.76% (15,987) with unknown diagnosis. National ambulatory cases in 2008-2010 comprised 64.72% (1,249) in government hospitals, 30% (579) in private hospitals and clinics, and 5.34% (103) unknown. National hospitalized cases in 2008-2011 included 63.36% (255,577) in government hospitals, 32.62% (131,590) in private facilities, and 4.02% (16,200) unknown. Case fatality rate is highest among infants of less than 1-year old followed by 1- to 10-year old children. This study is relevant in understanding the burden of dengue, its control program, and in estimating its economic cost in the country.

581

TEMPORAL VARIATION IN THE RELIABILITY OF PASSIVE SURVEILLANCE FOR ESTIMATING DENGUE INCIDENCE

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Dengue imposes a significant public health burden in tropical and subtropical regions. The full extent of its human and economic burden is not clearly defined, however, because surveillance systems routinely underrepresent total disease incidence. The extent of disease underreporting can be estimated by comparing case counts passively reported to local health authorities with those identified using more active and thorough methods, and calculating an expansion factor (EF). EFs can vary inter-annually, but observed variation in the accuracy of a single passive surveillance system for relative incidence over time has not been rigorously examined. We compared laboratory-confirmed dengue incidence in Iquitos, Peru, between tri-weekly door-to-door surveillance in longitudinal cohorts (study population approximately 4,300-9,000) with clinic-based surveillance in 11 health centers (intended to serve approximately 240,000 residents). Of the five years analyzed for this project, results from 2007-08 and 2008-09 demonstrate annual EFs of 3.1 and 4.9, respectively. Aggregate estimates, however, mask intra-annual seasonal variation that was as high as a weekly EF of 41.7 during the 2007-2008 peak in transmission. There was a positive linear relationship (0.077, 95% CI=[0.053-0.10], $p<0.001$) between cohort disease incidence and EFs over both years, so that as disease incidence increased, the accuracy with which clinic-based surveillance reflected this change decreased. Additional analyses assess the relationship between surveillance efficiency and epidemiologic factors such as circulating serotype and age stratification. Our results highlight the need for a clearer understanding of the relationship between passive surveillance data and disease incidence, which will better inform vector-control intervention decisions by public health authorities.

582

EVALUATION OF A DENGUE COURSE FOR PHYSICIANS IN PUERTO RICO

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Prior to 2010, the clinical management of dengue patients by physicians in Puerto Rico was not consistent with World Health Organization guidelines, as evidenced by chart reviews of fatal dengue cases in 2007 and a physician survey in 2008. A four-hour, classroom style course on dengue clinical management was developed and given to 7,813 physicians in 2010; this course was required for maintenance of licensure in Puerto Rico. We evaluated course effectiveness by measuring differences in patient care practices before and after the course. We reviewed 430 adult and 1075 pediatric medical records at the 12 hospitals in Puerto Rico with the highest number of reported lab-confirmed dengue inpatients during 2008-9 (before the course) and 2011 (after). A mixed-effects logistic regression with a random effect for hospital to account for anticipated within-hospital correlation was used to compare selected indicators of dengue management. The percentage of patients who did not receive corticosteroids increased for adult patients from 30% to 68% (OR 5.6, 95% CI 3.2-10.0) and for pediatric patients from 91% to 96% (OR 2.7, 95% CI 1.3-5.6). Usage of only isotonic intravenous saline solutions increased for adult patients from 49% to 69% and for pediatric patients from 9% to 16% (common OR 2.3, 95% CI 1.6-3.2). Ordering of fluid input and output monitoring increased for pediatric patients from 26% to 39% (OR 2.0, 95% CI 1.4-2.8) and for adult patients from 19% to 22% (OR 1.1, 95% CI 0.6-2.0). A statistically significant improvement in the management of dengue inpatients between 2008-9 and 2011 was detected in the hospitals with the most dengue patients, an effect that was likely due to the 2010 dengue course. Despite these significant results, improvement in clinical management is still needed for steroid usage in adults, isotonic fluids in children, and fluid monitoring in both groups. An online version of the course has been developed which should expand its reach and sustainability.

583

IDENTIFICATION AND GENERATION OF DENGUE VIRUS VACCINE CANDIDATES USING A VIRUS-LIKE PARTICLE PLATFORM

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Dengue viruses (DENV), which are members of the *Flaviviridae* family, comprise four distinct serotypes, called DENV-1, DENV-2, DENV-3, and DENV-4. Primary infection leads to lifelong protection against the infecting serotype, but subsequent infection by another serotype sometimes leads to potentially severe and life-threatening illness as a result of antibody-dependent enhancement (ADE) of infection by non-neutralizing, but cross-reactive, antibodies. Because non-neutralizing antibodies enhance pathogenesis, an ideal vaccine for DENV would direct the antibody response only to neutralizing epitopes, especially those shared by the four serotypes of DENV, but not non-neutralizing or partially neutralizing antibodies associated with ADE. We have developed a peptide display and affinity selection platform, which integrates epitope discovery capabilities of phage display with the high immunogenicity of virus-like particle (VLP) antigen presentation. We have constructed large (>10^{10}), diverse libraries of random peptides displayed on VLPs of the RNA bacteriophage MS2. We have shown that VLPs can be affinity-selected from these libraries using monoclonal antibodies (mAbs) and that selected VLPs can be used directly as immunogens to elicit epitope-specific responses. In this study, we performed affinity selections using a panel of broadly neutralizing anti-DENV monoclonal antibodies that recognize conformational epitopes in the EDIII or EDI/II domains of DENV E protein. These selections yielded a collection of VLPs displaying mimotopes of the viral epitopes and bound strongly to the selecting mAbs. VLPs were expressed, purified, and used to immunize mice. Collected sera will be assessed for neutralizing activity against all four DENV serotypes by a plaque reduction neutralization test using live virus or by dengue reporter virus particles. By targeting specific epitopes, our goal is to elicit specific high-titer responses against vulnerable domains of DENV, avoiding the complications associated with non-neutralizing and infection-enhancing antibody responses.

584

AN UNRECOGNIZED OUTBREAK OF DENGUE - ST. CROIX, U.S. VIRGIN ISLANDS, 2012

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In November 2012, a school nurse in St. Croix reported 27 suspected dengue cases among 369 (7%) students and staff to the Virgin Islands Department of Health and CDC. An investigation was begun to estimate dengue virus (DENV) infection rates in schools island-wide. Six randomly selected public schools participated in a stratified two-stage cluster serosurvey. Case finding for suspected cases was conducted at St. Croix's sole hospital to identify patients who had dengue serologic testing from January 2012-January 2013. Stored specimens from December-January were obtained and tested further by real-time RT-PCR at CDC. A dengue knowledge and prevention practices survey was sent to a representative sample of the parents of students at six elementary schools. Of 168 student and 91 staff specimens, 19% of students (95% CI 13%-25%) and 12% of staff (95% CI 4%-20%) were IgM anti-DENV positive, indicating infection in the previous 3 months; 3 students and no staff were PCR positive, indicating current infection. Of the remaining 134 students and 78 staff who were IgM- and PCR-negative, 77% of students (95% CI 62%-91%, mean age 10.5 years) and 96% of staff (95% CI 91%-100%) were IgG anti-DENV positive, indicating past DENV infection. Hospital case finding identified 308 total suspected cases, of which 209 (68%) were from December-January; of these 209, 104 (50%) were positive by IgM or PCR. Of 715 parent surveys returned, 69% of parents in St. Croix (95% CI 66%-71%) could not identify the DENV mosquito vector, 41% (95% CI 39%-44%) did not know that these mosquitoes are peridomestic, and 51% (95% CI 48%-55%) did not use insect repellent. This large dengue outbreak infected nearly one in five students. Anti-DENV IgG prevalence indicated that most children in St. Croix have been infected by DENV. Despite the apparent high rate of DENV transmission among St. Croix residents, families were not well informed about prevention. In places where dengue surveillance is challenging, the utility of schools as sentinels for seasonal outbreaks should be investigated.

585

CLINICAL AND LABORATORY RISK FACTORS OF DENGUE PATIENTS ADMITTED IN INTENSIVE CARE UNIT: A MATCHED CASE-CONTROL STUDY

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Dengue infection may result in severe clinical manifestations that required intensive care. However, there is limited knowledge on the clinical and laboratory risk factors of these dengue patients during first presentation at hospital. A retrospective hospital-based 1:4 matched case-control study was performed with 27 dengue patients admitted to the intensive care unit (ICU) from year 2004 to 2007, and 108 dengue patients who do not required intensive care matched by year of dengue presentation. Univariate and multivariate conditional logistic regression were performed. ICU dengue patients are significantly older (median age=44; P=0.003) and have diabetes (14.8%; P=0.031), compared to non-ICU dengue patients (median age=34; 2.8% diabetics). ICU dengue patients are presented in the hospital on median 3 days post fever (dpf), and progressed to DHF/DSS on median 4 days post presentation (dpp), and stayed in ICU for a median of 3 days. Among the 27 ICU dengue patients, there were 7 deaths that occurred on median 7 dpf and 3 dpp. The period of hospitalisation for ICU dengue patients is significantly longer (median 8 days;P <0.0001) as compared to non-ICU dengue patients (median 4 days). After adjusting for age and diabetes, haematocrit increased of more than 20% with thrombocytopenia (P=0.001), hypoproteinemia (P=0.037), signs of plasma leakage (P<0.001), hypotension for age (P=0.005), shock (P=0.006) and severe organ involvement (P=0.003) were found to be significantly more common in ICU dengue patients than non-ICU dengue patients. Furthermore, maximum pulse rate [adjusted conditional odds ratio(ACOR)=1.05;95% confidence interval(CI)=1.01,1.09], maximum neutrophils (ACOR=1.11;95%CI=1.04,1.17), urea (ACOR=1.26;95%CI=1.02,1.56) and creatinine level (ACOR=1.02;95%CI=1.01,1.04) were associated with higher risk of admission in ICU. These risk factors identified during first presentation may be useful in complementing the World Health Organization's severity classification of dengue patients that may further enhance clinical management of these high risk patients.

586

UTILITY OF WARNING SIGNS IN PREDICTING SEVERE DENGUE AND GUIDING ADMISSION

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The recommendation from the 2009 World Health Organization guidelines for managing dengue is to hospitalize patients with any warning sign for observation and management. We evaluated the utility of using warning signs to guide hospital admission and predict disease progression in adults. We conducted a prospective cohort study from January 2010 to September 2012. Daily demographic, clinical and laboratory data were collected from adult dengue patients. Warning signs were recorded. The sensitivity, specificity, positive and negative predictive values of warning signs in guiding hospital admission and predicting disease progression were evaluated. Four hundred and ninety-nine patients with confirmed dengue were analyzed. The sensitivity of warning signs in guiding admission for dengue hemorrhagic fever (DHF) II-IV and severe dengue (SD) was high but specificity was 52% and 47.5%, respectively. Having any warning signs had 100% sensitivity in predicting progression to DHF II-IV and SD but specificity was 52% and 48%, respectively. Absence of any warning signs had a NPV of 91%, 100% and 100% for DHF I-IV, DHF II-IV and SD. Of those who progressed to severe illness, 16.3% had warning signs on the same day while 51.3% had warning signs the day before developing severe illness. Our findings demonstrated that patients without any warning signs can be managed safely in ambulatory care to reduce burden on healthcare resources. No single warning sign can independently predict disease progression. The window from onset of warning sign to severe illness in most cases was short.

587

SELF-REPORTED PAIN INTENSITY USING THE NUMERIC REPORTING SCALE IN ADULT DENGUE MANAGEMENT

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Pain is a prominent feature of acute dengue as well as a clinical criterion in World Health Organization guidelines in diagnosing dengue. There is a paucity of data detailing the development of pain during acute dengue in different epidemiological groups and in relation to disease progression. We conducted a prospective cohort study in laboratory confirmed adult dengue patients managed at a tertiary infectious disease referral centre in Singapore using a self-reported 11-point Numeric Pain Scale to quantify and compare levels of pain during acute dengue between different age groups, gender, hospitalization status and dengue severity. Self-reported pain scores were measured at each daily clinic visit and analyzed using logistic regression. We also explored the use of pain score as an independent predictor of dengue severity. We found that 90% of patients reported pain during acute dengue with a trend towards greater reporting of moderate/severe pain in those below 55 years (68%) compared to those older (44%) though this did not reach statistical significance at the 5% level. We found no statistically significant differences in levels of pain reported by dengue patients stratifying by gender, hospitalization status, or disease severity. Highest pain scores were reported at days 5-7 of illness and diminished rapidly subsequently. Peak levels of pain did not reliably precede development of dengue hemorrhagic fever or severe dengue. Pain scores were not useful in predicting progression to severe disease.

588

THE DENGUE VACCINE INITIATIVE PROJECT: PASSIVE FACILITY-BASED FEVER SURVEILLANCE IN CHILDREN AND ADULTS OF SANTA CRUZ COMUNA OF MEDELLIN, COLOMBIA, AND BANG PHAE DISTRICT OF RATCHABURI PROVINCE, THAILAND

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Dengue fever (DF) is a major public health problem in Colombia and Thailand. Colombia experienced its largest epidemic with almost 157,000 DF cases and 217 deaths in 2010; Thailand reported provincial incidence rates of 698/100,000 person-years. For further evaluation of the burden of dengue, the Dengue Vaccine Initiative (DVI) is conducting fever surveillance in adults and children in Medellin, Colombia and in Ratchaburi province, Thailand, likely early adopter countries of a dengue vaccine. DVI is conducting a passive facility-based surveillance complemented by a healthcare utilization survey (HUS) to determine the burden of DF in Bang Phae district of Ratchaburi province in Thailand and Santa Cruz comuna of Medellin in Colombia. In the surveillance, every other eligible febrile patients between 1-55 years-of-age have been enrolled. We collected acute and convalescent blood samples to test for DF using NS-1 rapid test, IgM/IgG ELISA, followed by RT-PCR. From the HUS, we identify the proportion of febrile cases missed by the passive surveillance. The 1st year of fever surveillances launched in Oct. and Nov., 2011 in Bang Phae Community Hospital (BPCH) and Santa Cruz Hospital (SCH), respectively. There were 42 DENV positive cases among 349 subjects in BPCH and 15 lab-confirmed cases among 147 subjects in SCH. From BPCH, 34 and 8 patients were diagnosed as secondary and primary DF, respectively. Among 42 ELISA-positive cases, 32 cases were positive on RT-PCR, showing that DENV2 is the most commonly circulating serotype. From SCH, 8 and 7 individuals were diagnosed as secondary and primary DF, respectively. RT-PCR results are being processed. Almost 30% of the individuals with recent fever reported to not seek care through our facilities. Thus, the incidence rates are 96.9/100,000 and 601/100,000 person-years for Santa Cruz and Bang Phae, respectively. More data will be available for presentation. he incidence rates for DF calculated were almost 100/100,000 and 600/100,000 person-years for Medellin and Ratchaburi, respectively. Epidemiologic data, in addition to other economic and private demand data collected, will be used as evidence for decision-making for dengue vaccine introduction in Thailand and Colombia.

589

REGULATION OF THE ANTIOXIDANT DEFENSE IN MOSQUITO CELLS INFECTED BY DENGUE 2 VIRUS

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Dengue viruses (DVs) generally cause trivial deleterious effects in mosquito (C6/36) cells, in contrast to what occurs in mammalian cells. As superoxide was detected in C6/36 cells that had been infected with DV-2, suggesting that oxidative stress may occur in those cells. Nevertheless, antioxidant defense is actually elicited, leading to protection of the mosquito cells from DV infection. To understand the regulatory mechanism in association with elicited antioxidant genes, we have identified a novel *p53* from C6/36 cells infected by DV-2 for 24 h. Although the *p53* was classified as a tumor-suppressor gene in mammalian cells, we have recently proved that it has an alternative function involving in antioxidant defenses of DV-infected mosquito cells. Knockdown of the *p53* gene in DV-9 in infected

C6/36 cells with a specific synthetized dsRNA, both the superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) significantly increased at 48 hpi. Moreover, the cell death rate raised to 8.49% compared to those without knockdown of the *p53*-like gene. Among antioxidant genes tested in this study, catalase was identified to be specifically regulated by the *p53*-like gene. Furthermore, ROS concentration in DV-infected cells was shown to be involved in regulation on the cell death rate. It seems that catalase is one gene regulated by *p53*-like gene and involved in antioxidant defense of mosquito cells in response to DV infection, presumably dependent on reduced ROS accumulation.

590

UPDATE ON THE DEVELOPMENT SANOFI PASTEUR RECOMBINANT CYD TETRAVALENT DENGUE VACCINE

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Results in 2012 from a Phase IIb efficacy trial of the CYD tetravalent dengue vaccine in the Ratchaburi province of Thailand, showed for the first time that a safe, efficacious vaccine against dengue is possible. It also raised questions on the reference PRNT assay methods, challenged some of the fundamental dengue vaccine development hypotheses, and served as a reminder of the complexity of dengue disease. Analyses have been carried out and are still ongoing to understand the main finding from the PhIIb study (that although efficacy was seen for 3 serotypes, none was seen for DENV2, despite satisfactory PRNT titers). These analyses included the sequencing of the clinical isolates from the PhIIb study, which, despite differences with the vaccine, were nevertheless cross neutralized in Vero cell-based *in vitro* assays In parallel, investigations continue to characterize vaccine-induced cellular responses: analyses from a trial in Singapore have confirmed the previous data showing the induction of broad serotype-specific Th1 responses dominated by IFNγ; in addition, ongoing long-term follow up shows that cellular reponses persist for at least one year after vaccination. Assessment of safety remains a critical component of the dengue vaccine program. As of January 2013, more than 28,900 have received one or more CYD-TDV vaccinations, with no safety signals identified in ongoing safety surveillance. A formal integrated safety analysis of 9 completed clinical trials with more than 5300 vaccinees confirms the good reactogenicity and safety profile seen in individual studies, including in the PhIIb study with 2 years of active follow-up. In the pivotal phase III efficacy trials, vaccinations have been completed and active surveillance is ongoing, and the drop out rate after 20 months is <5%. This low proportion attests to the considerable site preparation efforts by the local teams and the investigational teams' commitment, and illustrates the importance of dengue disease for the communities. The results of these phase III trials are expected by the end of 2014.

591

SAFETY OF THE CYD DENGUE VACCINE: INSIGHTS FROM AN INTEGRATED ANALYSIS OF 5,344 INDIVIDUALS VACCINATED IN NINE CLINICAL TRIALS

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The live, attenuated CYD tetravalent dengue vaccine (TDV) is in phase 3 evaluation. We analyzed pooled safety data from all 9 trials completed to Aug. 2012 evaluating a 0-6-12 month schedule. In these trials 15433 CYD-TDV doses were received by 5344 participants, and 2175 received placebo. The overall SAE reporting rate was comparable in vaccines and placebo controls, and only 3 SAEs among vaccinees were considered as vaccine-related (headache [n=2], polymyalgia rheumatica). In all, 5 severe dengue cases have been documented: 2 among vaccinees and 3 among controls. Among adults, 12-17 year-olds and 2-11 year-olds, after any injection: solicited injection site reactions (SISR) were reported for respectively, 51.2%, 45.6%, 59.0% with CYD-TDV, and 16.8%, 32.4%, 48.5% with placebo, while solicited systemic reactions (SSR) were reported for 69.8%, 69.4%, 69.5% after CYD-TDV, and 44.5%, 59.5% and 57.2% after placebo, respectively. SISRs were reported at comparable rates after each dose and were most commonly injection site pain. SSRs were less frequent after 2nd and 3rd injections than after the 1st and were most commonly headache, malaise and myalgia. Solicited reactions were mild to moderate in almost all cases, resolving typically within 3 days. Unsolicited adverse events were injection site reactions, gastro-intestinal disorders and infections, and were assessed as vaccine-related for 7.2% of vaccines, and 2.8% of placebo controls. In this dataset, there was no increase of reactogenicity after successive doses, no marked difference in the safety profile between flavivirus seropositive and seronegative individuals at baseline, no serious allergic reactions, neurotropic or viscerotropic disease, and no excess of severe dengue cases in vaccinees compared to controls. All available clinical trial data therefore show CYD-TDV to have satisfactory safety profile, with no evidence of sensitization to severe dengue. Potential safety risks are carefully monitored in all ongoing trials, including a review by an Independent Data Monitoring Committee.

592

LABORATORY INVESTIGATION ON RE-EMERGENCE OF DENGUE FEVER IN MOMBASA, 2013

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Since the last quarter of the 20th century, frequency of dengue epidemics has significantly increased in many tropical regions including several locations in Africa. The global incidence is currently estimated at 50-100 million cases reported annually from over 100 countries. In March 2013, samples from 2 febrile patients from a Mombasa hospital were sent to the Viral Hemorrhagic Fever laboratory at the Kenya Medical Research Institute/Walter Reed (KEMRI/WRP). Testing was conducted to rule out arboviruses or viral hemorrhagic fevers by ELISA and RT-PCR. Both samples tested positive for dengue virus by RT-PCR, specifically dengue serotypes 1 and 2 (DEN1, DEN2); however, they were negative by IgM ELISA. Passive surveillance was implemented at 7 local clinics/hospitals, which consisted of administration of a questionnaire to suspect cases including demographic and clinical data and collection of a blood sample. From March to April 2013 a total of 185 samples were collected. The samples were tested for exposure to dengue by IgM ELISA and RT-PCR at KEMRI/WRP and CDC laboratories in Nairobi, Kenya. The age range of the patients was 3-75 years. The majority of cases were male (54%). A total of 21/185 (11.3%) samples tested positive by IgM ELISA, while 60/185 (32.4%) were positive by RT-PCR. Serotyping results showed the following serotypes circulating in Mombasa, DEN1 33/60 (55%), DEN2 17/60 (28.3%), DEN3 4/60 (6.6%) and two samples had dual infections of DEN1 and 3. The current investigations have shown that multiple dengue viruses are circulating in Mombasa since the last confirmed dengue outbreak of 1982. Sequencing and phylogenetic analysis of the isolates will shed more information on the evolutionary patterns of the Mombasa dengue viruses.

593

SEQUENCE ANALYSIS OF THE DENV2 STRAINS ISOLATED IN THE PHASE IIB CYD VACCINE EFFICACY TRIAL IN RATCHABURI, THAILAND

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A phase IIb clinical trial was conducted in the Ratchaburi province of Thailand with the tetravalent CYD vaccine. The observed lack of protection against DENV2 led to further exploratory analyses. We sequenced isolates from dengue cases on prM to E coding regions using PCR amplifications with serotype-specific primers designed to cover all known dengue strains. In all, 17 DENV1, 53 DENV2, 3 DENV3 and 4 DENV4 samples were sequenced. DENV1, 2 and 4 samples were each identified as belonging to single virus lineage. There were 3 DENV3 sequences in two distinct genotypes. Phylogenetic analyses show no relationship between efficacy, and the genetic distance between circulating and vaccine strains. Indeed, DENV3 and 4 genotypes differed from that of the vaccine but showed protection, in contrast to DENV2 for which the circulating virus and the vaccine' parental strain were of the same Asian I genotype. However, significant differences exist within this genotype, and the DENV2 strain circulating in Ratchaburi during the clinical trial had been described in 2010 in Vietnam as a new, and rapidly emerging strain, causing higher viremia in humans. One specific amino acid signature of that strain is E-83-226-228-346 (KKEY), which is different to the vaccine's parental DENV2 strain PUO 218. Mapping these amino acids on 3D models shows that the first 3 residues are close to each other on domain II, and easily accessible at the surface. Data in Genbank show that this specific profile consistently circulates in only three countries (Vietnam, Thailand, Cambodia) of continental South-East Asia. No such profile was identified in America, India, Carribean, Pacific, Africa or non-continental Asia. While no immune escape was seen for that lineage in Vero-cell based neutralization assays using vaccinee's sera, it cannot be excluded that circulating virus is able to overcome vaccine-induced immunity due to high *in vivo* fitness. Phase III efficacy trials in countries with and without this lineage of DENV2 will help understand the Thai phase IIb efficacy trial results.

594

DENV REPORTER VIRUS PARTICLES AND THEIR UTILITY FOR CLINICAL AND FUNCTIONAL APPLICATIONS

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The lack of reliable, high-throughput tools for characterizing anti-dengue virus (DENV) antibodies in large numbers of serum samples has been an obstacle in understanding the impact of neutralizing antibodies on disease progression and vaccine efficacy. In the current study, we demonstrate the diagnostic utility of DENV RVPs for measuring neutralizing antibodies in human serum samples against all four DENV serotypes, with attention to the suitability of DENV RVPs for large-scale, long-term clinical studies. DENV RVPs are antigenically equivalent to live virus, show serotype-specific responses against human sera, and yield reproducible neutralization titers that are in statistical agreement with PRNT results. In addition, using a technology called Shotgun Mutagenesis, we created a comprehensive plasmid mutation library for DENV-3 prM/E, in which each amino acid was substituted. DENV RVPs containing each prM/E variant were produced and assayed for viral budding and infectivity in order to identify residues critical for infectivity. Critical residues were mapped and visualized on crystal structures for prM and Env. Taken together, DENV RVPs offer advantages for detecting immune responses with application to large-scale clinical studies of DENV and can be used for understanding important structure function relationships for DENV Env protein.

595

THE DENGUE VACCINE INITIATIVE PROJECT IN COLOMBIA AND THAILAND: SERO-PREVALENCE STUDY IN CHILDREN AND ADULTS OF SANTA CRUZ COMUNA OF MEDELLIN AND BANG PHAE DISTRICT OF RATCHABURI PROVINCE

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Dengue infection is a major public health problem in both Colombia and Thailand, likely early adopters of dengue vaccines. Colombia experienced its largest epidemic with almost 157,000 DF cases and 217 deaths in 2010 and Thailand has reported provincial incidence rate up to 698/100,000 person-years. Often sero-prevalence data are not available and estimation of the overall disease burden is incomplete due to inaccurate capture of asymptomatic dengue infection cases in both adults and children. In preparation for the upcoming dengue vaccine, the Dengue Vaccine Initiative is conducting sero-prevalence study, linked to fever surveillance, in Colombia and Thailand. The Dengue Vaccine Initiative Project (DVI) is conducting a sero-prevalence study to determine the burden of inapparent dengue infection in Bang Phae district of Ratchaburi province in Thailand and Santa Cruz comuna of Medellin in Colombia. From randomly selected residents between 1-55 years-of-age in the catchment area population, we collect 2000 paired sera with 6 months interval to estimate age-specific sero-conversion rate. To evaluate dengue immunity status, we perform PRNT on those samples that show rise in the IgG ELISA. In the 1st year of the sero-prevalence study, 2012 and 2009 subjects were recruited in Bang Phae district and Santa Cruz comuna, respectively. As of April 2013, the in-house IgG ELISA testing is complete for the paired sera collected from Bang Phae and ELISA/PRNT data will be available for presentation at the conference. From 2009 paired samples collected from Santa Cruz comuna, the overall sero-conversion rate of 5.1% was found across the age-group. The highest sero-conversion rate (7.0%) was found among the children with 5-9 years of age, followed by those 35-44 years-of-age with 6.0% sero-conversion rate. From preliminary PRNT performed on a subset of sero-converted samples (n=118), only 1 sample showed monovalent response to DENV 4. Almost 74% (n=87) showed immune response against all 4 serotypes. The sero-prevalence data generated will complement clinical data from the fever surveillance, as well as other economic, behavioral, private demand data, to provide essential evidence for decision-making for vaccine introduction in Thailand and Colombia.

596

DETECTION OF NS1 PROTEIN IN ACUTE PHASE SERUM SAMPLES IS NOT ENOUGH TO MAKE THE DIAGNOSIS OF DENGUE-4 INFECTIONS IN BRAZIL

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Dengue is an acute febrile illness resulting from the infection by any of the four dengue virus serotypes and affecting about 100,000 people annually worldwide. Ribeirão Preto city, in Brazil, has experienced several large dengue outbreaks. Due to this fact, a virus surveillance study was implemented to determine the serotypes circulating in the region and to start control measures as soon as possible. With this approach, we have been able to map the dengue serotypes circulating each year and to predict their alternance in causing the outbreaks. As an example, we detected that dengue-1 virus (DENV-1) was the predominant circulating serotype for the last three years but dengue-4 virus (DENV-4) had started

to circulate in the city late in the last year. The virus surveillance approach consists of sending to our lab about 20 acute phase samples per week, equally distributed from the city and randomly selected from city reference health centers. Samples were collected from patients with dengue-like symptoms. In the lab, samples were tested by NS1-ELISA, IgG- and IgM-capture ELISAs, according to manufacturer's instructions (PanBio, Queensland, AU), and then, by polymerase chain reaction (PCR). From late December of last year to early April of 2013, 223 samples were sent to the lab. Evaluation by the NS1-ELISA showed that 71 samples were positive, 18 were inconclusive, and 134 were negative. PCR results showed that out of 71 NS1-positive samples, 56 were positive for DENV-4 and 15 for DENV-1, and among the inconclusive samples, 15 were DENV-4-positive and one was positive for DENV-1. However, an interesting finding was that when analyzing the 134 NS1-negative samples, 72 were positive for DENV-4 and none for other dengue serotypes. It was clear that the NS1-ELISA was not adequately detecting the DENV-4 NS1 protein and one of the reasons for this finding was that most infections occurring in the city were secondary infections, resulting in the observed low-level NS1 detection. However, among those 134 NS1-negative samples, 94% of them were IgG-negative, showing that most of patients from whom the samples were collected were having primary infections. Thus, our results show that DENV-4 NS1 protein has not been adequately detected by commercial tests resulting in inadequate diagnosis of dengue-4 infections.

597

INHIBITION OF CHOLESTEROL SYNTHESIS AND ITS EFFECT ON DIFFERENT PHASES OF DENV REPLICATION CYCLE

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Although antibody dependent enhancement (ADE) effect and immunology related factors have been appointed as causative for severe Dengue, several cases are not consistent with this hypothesis, suggesting that other metabolic molecules could be involved. It has been reported that cholesterol levels are regulated by DENV infection *in vitro* and that lipids are necessary for virus replication. In addition, Lovastatin (LOV) treatment reduces viral RNA on a DENV replicon system. The aim of this work is to evaluate the antiviral profile of three statins against DENV at different phases of the viral replication cycle.HuH-7 Hepatoma cells were infected with DENV at a MOI of 4. Cells were pretreated 6h pre-infection or treated 12h post-infection with LOV (5μM), Pravastatin (PRA) (5μM-10μM)) or Atorvastatin (ATO)(5μM). On a subset of cells, statin concentration was maintained by applying treatment every 12h. All cells were collected 48h post infection. Viral effect was measured by cytopathic effects detected and viral titter was measured by using the Plaque formation unit (PFU) assay in BHK-21 cells. A reduction in DENV infective particles was observed on all statin treated cells compared with the untreated control. DENV inhibition percentages for LOV, PRA and ATO were 50%, 20% and 70% respectively. 6h pre-treatment with statins slightly reduced virus titter while 12h post-infection continuous treatment result on a marked reduction on DENV infective particles up to 30% for PRA, 90% for LOV and 100% for ATO. A 12h post-infection treatment reduced viral titter more than the single pre-treatment dose but did not reach the inhibition levels of the post-infection continuous treatment. Our results indicate that inhibition of *de novo* synthesis of intra-cellular cholesterol results on reduction of DENV infectious particles and increased cytopathic effect. Furthermore, a higher inhibitory effect was observed when treatment was applied on latter stages of the replication cycle (post-infection single dose) and even more when treatment was maintained (post-infection continuous treatment). ATO showed the highest antiviral effect, which can be due to its larger half-life (14h). On the other hand, PRA showed the lowest effect, which can be related to its low lyposolubility.

598

COMPREHENSIVE MUTAGENESIS OF PRM/E TO IDENTIFY AND CHARACTERIZE EPITOPES ON DENGUE VIRUS

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Shotgun Mutagenesis technology was used to identify high-resolution epitope maps for dozens of human antibodies targeting the immunodominant envelope protein (prM/E) of Dengue virus (DENV). Comprehensive plasmid mutation libraries for DENV-3 and DENV-4 prM/E, comprised of over 2000 individual mutant clones, were created in which every prM/E residue was individually mutated to a defined substitution, expressed in human cells, and analyzed for its effect on antibody reactivity and viral infectivity. The neutralizing human anti-DENV monoclonal antibodies (MAbs) used in our studies were derived from infected patient B-cells and therefore represent a significant protective response of the human immune system. For each MAb, we identified amino acids on prM/E that are required for antibody binding, and these residues were mapped onto prM/E crystal structures to visualize and compare epitopes. Our goal is to map epitopes on DENV prM/E, determine their role in viral protection and pathogenesis, and how they relate to protein function. The binding kinetics of many of the MAbs has also been measured using biosensor binding to intact DENV virions. We expect that our work will help define the range of immunodominant structures on DENV prM/E and identify novel neutralizing antibody epitopes that can be used for improved therapeutics, diagnostics, and vaccine development.

599

NEUTRALIZATION OF WILD-TYPE DENGUE VIRUS ISOLATES BY ANTIBODIES ELICITED AFTER IMMUNIZATION WITH A TETRAVALENT DENGUE VACCINE

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A Phase IIb efficacy trial (Clinicaltrials.gov NCT00842530) of the CYD tetravalent dengue vaccine in the Ratchaburi province of Thailand was recently completed. Results indicated efficacy against symptomatic dengue caused by dengue virus (DENV) serotypes 1, 3, and 4, but none against serotype 2, despite measurable neutralizing antibody (NAb) responses, as determined by a validated PRNT50 assay. We sought to investigate whether circulating DENV strains had escaped vaccine-induced neutralization responses. We assessed NAb responses by a PRNT50 assay against the vaccine parental strains and circulating strains isolated during the study. Seven (7) isolates were selected based on sequence and phylogenetic analysis, and successful in-vitro amplification: 2 DENV-1, 2 DENV-2, 2 DENV-3, and 1 DENV-4. The optimal virus working dilutions and days post-infection were assessed to ensure accurate assignment of NAb titers with the circulating strains. In accordance with the protocol, sera drawn 28 days post dose 3 (PD3) of 284 participants, and from 74 dengue cases occurring after this time point were tested for NAb responses against vaccine parental strains. Exploratory testing for NAb responses against circulating strains was performed using PD3 sera from 45/284 participants and confirmed dengue serotype cases. Similar NAb titers were seen against the vaccine parental strains and recent circulating DENVs from Thailand. There was no lack of response against circulating strains, and neutralization of DENV2 did not show a different pattern to that observed against the other serotypes. The PD3 sera drawn prior to infection were able to neutralize the circulating strains in the Vero cell based PRNT50 assay. Given these data, it does not appear that circulating DENV strains escaped neutralization as measured by PRNT50 assay, and therefore does

not explain the efficacy trial results. Investigations are ongoing into the apparent contradiction between presence of neutralizing responses against serotype 2 and lack of efficacy.

600

ASSOCIATION OF POLYMORPHIC VARIANTS IN TNF-ALPHA, IL-6, RECEPTOR FCΓRIIA AND VITAMIN D GENES IN HONDURANS' WITH DENGUE INFECTION

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Dengue is an important problem of health public in tropical and sub-tropical countries. On the other hand, the response to dengue infection is influenced by the genetic background of the host. To evaluate the association of polymorphic variants in TNF-alpha 308, IL-6, Receptor FCγRII A and Vitamin D between individuals with different dengue disease presentation. The study was carried out in Tegucigalpa, Honduras, Central America. The study population consisted of 150 participant: 50 dengue fever (DF) and 50 dengue hemorrhagic fever (DHF) plus 50 asymptomatic controls (AC), all of them with proven dengue infection. The dengue classification was done following the WHO criteria. The selected single-nucleotide polymorphisms (SNPs) were carried out by a Real Time PCR methodology, using sequence specific primers, obtaining the allelic genotype for each investigated genetic marker. Comparisons between three population groups showed in DHF there was significantly more allelic frequency of TNFa-308 genotype GG (OR= 7.76, p= 0.0006) than in the AC, more over the frequency of this genotype is proportionally higher as disease severity progresses (AC: 67%, DF: 86% and DHF: 94%); genotype AA is absent in both DC and DHF. For FcgRIIA-131 H/R and IL-6-174 we observed no differences in the different population groups. As for the VDR-352, there is a higher frequency for the homozygous genotype TT(40%) in DF compare with DHF and AC (27 y 22% respectivetly), there is a tendendy for this polymorphism can be implied in protection to the infection severity for dengue virus for this genotype(TT) (OR=0.43, p=0.0597). These results provide evidence for the first time in Honduran population about the genetic susceptibility to the infection for dengue virus. However further studies are still necessary.

601

DEFINING EARLY TRANSCRIPTIONAL SIGNATURES OF THE IMMUNE RESPONSE TO A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE (DENVAX) IN NON-HUMAN PRIMATES

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The mechanisms by which vaccines induce a strong and diverse immune response for generating long-term protective immunity remain largely unknown. High-throughput transcriptional profiling has the advantage of providing insight into the features of an early immune response to vaccination that cannot be observed using standard immunological assays. In this study, we sought to measure the early transcriptional responses in cynomolgus macaques following vaccination with the tetravalent dengue vaccine DENVax, by different methods and routes of vaccine administration. Six groups of animals were administered DENVax by different routes (intradermal or subcutaneous), methods (needle and syringe or PharmaJet injector), and dose (single or two injections on day 0). A control group received PBS. DENVax induced an early transcriptional response in non-human primates that peaked on day 3 post-immunization. The molecular signature associated with DENVax is characterized by networks of significantly up-regulated genes involved in interferon signaling (p=4.71E-09) and the antiviral response (p=5.08E-06), and is similar to that seen in humans vaccinated with other live attenuated viruses, including YF17D and live attenuated influenza virus. The magnitude of this early interferon response was greater following subcutaneous administration than intradermal; similarly, animals inoculated subcutaneously also had stronger humoral responses. Two injections on day 0 elicited a greater number of significant changes in gene expression than a single dose, but up-regulation of key genes involved in interferon signaling and apoptosis were observed with both dosing schemes. Method and route of DENVax administration resulted in subtle differences in gene expression over time. These data suggest that the strength of the early immune response to DENVax may play a role in the magnitude of the subsequent adaptive response.

602

IDENTIFICATION OF FUNCTIONALLY CRITICAL REGIONS OF DENGUE VIRUS ENVELOPE PROTEIN

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Our current understanding of the role of envelope protein E in mediating Dengue virus (DENV) infectivity is supported by structures of E in different infective states, yet we lack a detailed mechanism for the final stages of infection, where low pH triggers E structural transformations and interactions to promote virus-host membrane fusion. By generating reporter virus particles (RVPs) from a comprehensive DENV-3 prM/E mutation array so that each residue in the polyprotein is individually mutated and tested for ability to mediate fusion, we have identified key residues in E that are critical for viral infectivity. The locations and interactions of these critical residues explain how DENV E functions on an atomic level. These residues fall into 5 distinct functional groups that are proposed to: (1) provide fusion loop protection prior to triggering or post-triggering fusion loop support to stabilize the E fusion trimer, (2) enable hinge movements that occur between the E domain interfaces during structural transformations, (3) mediate the formation and triggered disruption of interactions between E and protein M, and ultimately enable "zipper" contacts with E stem region to drive membrane fusion, (4) facilitate formation and triggered disruption of contacts between E ectodomain and stem, and enable crucial zippering interactions that promote virus-host membrane fusion, or (5) provide a strong membrane anchor for the fusogenic E trimer, by means of cross-helix interactions between transmembrane regions E-T1 and E-T2. Our studies reveal novel details of the DENV fusion pathway by identifying molecular interactions and roles in structural transitions for key functional residues whose actions are critical for E dimer stability, the dynamic processes of pre-fusion structural triggering, and fusion of virus and host membranes.

603

THE CARIBBEAN CONNECTION AND THE RE-EMERGENCE OF DENGUE IN EUROPE

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Dengue is caused by 4 different but antigenically related viruses, DENV-1 to DENV-4, transmitted to humans through the bites of *Aedes* mosquitoes. The disease is endemic in 100 countries from Asia, America, Africa and Oceania. In Europe, in the past century, Dengue epidemics occurred in Greece and other Mediterranean countries were the incriminate vector

was *Ae. aegypti*. After that, it disappeared from Europe, but another competent vector, *Ae. albopictus*, was introduced in late 70s.In recent years, *Ae. aegypti* was reintroduced in south Russia and in Madeira island (Portugal). In 2010, dengue re-emerged in the French Riviera and Croatia, with small outbreaks. Two years later, in October 2012, a sustained and explosive epidemic appeared in Madeira Island. Both, 2010 and 2012 outbreaks were caused by DENV-1.Travellers from different European countries acquired the infection in the tropics and carried the virus to areas were the vector is present. One viremic traveler could introduce the virus and initiate an autochthonous transmission of dengue in non-endemic area. The objective of this study was to describe the phylogeny and phylogeography of the DENV-1 introduced in Europe in the recent outbreaks. We analyzed complete E sequence from imported dengue infections acquired by returning travelers from The Caribbean and Latin American countries. Phylogenetic analysis revealed that all DENV-1 strains belong to genotype V. The strains introduced in France and Madeira clustered within different South American lineage. In conclusion, our data suggest that 2 different introduction of American DENV-1 occurred in Europe and were the responsible for the outbreak observed in 2010 in France and the epidemic of 2012-13 in Madeira, island.

604

ASSOCIATION OF POLYMORPHISMS IN FCΓRIIA AND DC-SIGN1 WITH THE CLINICAL PRESENTATION OF DENGUE INFECTION IN A MEXICAN POPULATION

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Dengue is the world's most prevalent vector-borne viral disease. The dengue virus causes a spectrum of illness including asymptomatic infection, a mild febrile illness, and in a small portion of cases dengue hemorrhagic fever or dengue shock syndrome. In the state of Morelos, Mexico, dengue infection is an increasing problem, with 488.2 reported cases per 100,000 habitants in 2008. Two SNPs, rs1801274 of FcγRIIa and rs4804803 of DC-SIGN1, have previously been associated with resistance or susceptibility to severe dengue infection in addition to other infectious diseases. Both of these polymorphisms are located in genes which code for receptors with important roles in dengue pathogenesis, and the relationship between these SNPs and clinical dengue infection in Mexican populations is unknown. In this study, real-time PCR was used to characterize the distribution of rs1801274 and rs4804803 in subjects with asymptomatic dengue infection (n=145), dengue without complications (n=64), and severe dengue (n=35) in Morelos. In contrast to previous studies, the arginine (G) variant of rs1801274 was associated with greater clinical severity of dengue. Homozygotes for the arginine variant were significantly more likely to present symptomatic (uncomplicated or severe) infection compared with asymptomatic infection (p=0.027). The frequency of the arginine allele was also significantly associated with symptomatic infection (p=0.026). The G variant of rs4804803 was found to be very rare in the population of study, with a frequency of only 5.17%, and was not significantly associated with the clinical presentation of dengue infection. Logistic and ordinal regression models relating the SNPs to severity of infection were also generated, accounting for covariates including primary or secondary infection and other environmental variables. These findings demonstrate the variability and complexity of factors involved in the development of severe dengue infection. Gene interactions may explain why the effects of the rs1801274 FcγRIIa polymorphism differ between populations.

605

VIRAL KINETICS OF PRIMARY DENGUE VIRUS INFECTION IN NON-HUMAN PRIMATES: A SYSTEMATIC REVIEW AND INDIVIDUAL POOLED ANALYSIS

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Viremia kinetics directly influence the clinical course and transmission dynamics of dengue virus (DENV). Despite a handful of studies examining DENV viral kinetics *in vivo*, the majority of the within-host dynamics are unknown. Significant ethical barriers prevent experimental studies in humans, and as such, non-human primates have been used as a model system for DENV infection for decades. In the current work we identify papers with experimental DENV infection in non-human primates and employ survival analytic techniques to estimate the time to viremia and duration of viremia as well as use mixed-effects models to assess associations between these and serotype, inoculating dose, viremia assay, and species of primate. We estimate that the median time to viremia in rhesus macaques (the most numerous species used) ranges from 2.63 to 3.32 days for DENV-2 and -1, respectively and that the median duration ranges from 3.13 to 5.13 days for DENV-4 and -2, respectively. We find no significant differences between species of primate for either time to viremia or duration; the time to viremia for DENV-4 is significantly longer than for DENV-2 and duration of viremia is significantly shorter for DENV-4 than DENV-1 and -2. When viremia was assayed by reverse transcription PCR (RT-PCR), time to viremia was significantly shorter and duration of viremia was significantly longer than with plaque-forming assays. Finally, a significant negative relationship exists between inoculating dose of virus and duration of viremia. Knowledge of the within-host viral kinetics of DENV in non-human primates will aide in understanding the transmission dynamics of sylvatic DENV in populations of non-human primates, an issue of growing importance as dengue vaccines become available.

606

EARLY TRANSCRIPTIONAL RESPONSES THAT CORRELATE WITH NEUTRALIZING ANTIBODY DEVELOPMENT IN DENGUE VACCINE RECIPIENTS

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Dengue virus (DENV) is the most common mosquito-born virus worldwide, causing an estimated 400 million infections annually. Neutralizing antibodies elicited after infection play an important role in protection from subsequent infection with the homologous serotype, and development of neutralizing antibodies has been the primary endpoint for evaluating the immunogenicity of candidate dengue vaccines. However, little is known about early host responses following vaccination that lead to development of neutralizing antibodies, or to differences in the titer of neutralizing antibodies among individuals. To characterize the acute host response to dengue vaccination and identify early correlates of adaptive immune responses we examined the genome-wide transcriptional response to a live, attenuated dengue vaccine, DEN3Δ30/31, the DENV-3 component in the TV-003 tetravalent dengue vaccine candidate developed by NIAID. We analyzed longitudinal whole blood RNA samples (n=165) collected prior to vaccination, 7 times during the next two weeks, and on days 21,

28, 42 and 180 from 10 DEN3Δ30/31 vaccinees and 4 placebo recipients. Significant increases in transcript abundance after vaccination were evident between days 5 and 14 in 9 vaccinees who developed positive neutralizing antibody titers; the most notable feature was a wave of interferon-stimulated gene (ISG) expression that peaked between days 6 and 12 (median, day 9). ISG transcript abundance on day 8 correlated with antibody titer (PRNT60) measured on day 42 post-vaccination (Spearman's rho = 0.73). The single, non-responding vaccinee did not develop an ISG expression response following vaccination. A second set of transcripts with peak expression between days 12 and 20 was enriched for genes associated with lymphocyte proliferation and activation (p<1E-10). These findings suggest that it is possible to identify early correlates of protective adaptive immune responses soon after dengue vaccination, and provide a pathway for further understanding the cellular and physiological events leading to development of neutralizing antibodies.

607

PHYLOGEOGRAPHY AND MOLECULAR EPIDEMIOLOGY OF AN EPIDEMIC STRAIN OF DENV1 IN SRI LANKA

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In 2009, a severe dengue epidemic occurred in Sri Lanka that caused higher mortality and morbidity than any previously recorded epidemic in the country. In each subsequent year, dengue has continued to reach epidemic proportions, posing a major clinical burden to the population. The 2009 epidemic correlated with a shift in the predominant disease-causing dengue virus serotypes in Sri Lanka: prior to the epidemic, two serotypes, DENV2 and 3, were isolated from the majority of patients presenting with serious dengue disease, however, in 2009, a previously undetected DENV1 strain dominated as the major causative agent of dengue disease, and DENV1 has persisted as the dominant serotype in Sri Lanka. We amplified dengue virus from sera of patients who presented with severe disease to Colombo North Teaching Hospital in Sri Lanka during the Spring and Summer of 2012, and sequenced the full genomes of several DENV1 isolates. We report phylogenetic evidence that the 2009 epidemic DENV1 strain has continued to circulate within the population and was a causative agent of severe disease in Colombo, Sri Lanka during the 2012 epidemic. We applied bayseian phylogeographic methods to infer the historic spatial dispersion of this virus, using our Sri Lankan virus isolates and other reported sequences in the literature. These analyses suggest that the 2009 Sri Lankan epidemic DENV1 strain may have traveled directly or indirectly from Thailand, through China, to Sri Lanka, and, after spreading within the Sri Lankan population, traveled to Pakistan and Singapore. Our findings delineate the dissemination route of a virulent DENV1 strain in Asia and are of particular importance to global control efforts.

608

CO-INFECTION WITH DENGUE AND RESPIRATORY VIRUSES AMONG CHILDREN WITH ACUTE FEBRILE ILLNESS, PUERTO RICO

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Dengue is endemic in Puerto Rico with seasonal increases in incidence that often co-inside with increases in other acute febrile illness (AFI) due to viral respiratory pathogens. This can lead to difficulties in clinical diagnosis and delayed recognition of dengue or these other AFIs. A sentinel enhanced dengue surveillance system site in a tertiary care hospital in Ponce, Puerto Rico began conducting AFI surveillance in May 2012. Outpatients with fever or history of fever for <7 days were enrolled with informed consent and followed through their illness. Specimens including serum and nasopharyngeal swabs were collected and tested by RT-PCR and immunodiagnostic methods as appropriate for a number of pathogens including 4 dengue viruses (DENV-1-4), influenza A, influenza B, and 12 other respiratory viruses including adenovirus, respiratory syncytial virus, metapneumovirus, and parainfluenza viruses 1-4. From May 2012 through January 2013, 18 PCR positive co-infections with DENV and a respiratory virus were identified among the 1439 enrolled case-patients. Most (91%) case-patients with co-infections were children and adolescents <18 years of age. When compared with 398 DENV positive only case-patients, co-infected case-patient reported runny nose and cough 2.4 and 3.0 times more frequently, but this was not statistically significant. Nevertheless, chest x-rays were ordered 7 times more frequently in co-infected case-patients as compared to DENV only case-patients. No other symptom or sign, risk factor, or laboratory test was associated with co-infection. The overall prevalence of co-infections is low. Additional studies should be performed to evaluate outcomes associated with severity. However, in concurrent epidemics of dengue and respiratory pathogens, physicians must have a high index of suspicion of co-infection.

609

VACCINATION OF NON-HUMAN PRIMATES WITH DENVAX ELICITS SEROTYPE-SPECIFIC CD4$^+$ AND CD8$^+$ T CELLS WITH A PROINFLAMMATORY CYTOKINE PROFILE

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Dengue viruses have a significant impact on global health in the tropics and subtropics, particularly in Asia and Latin America. Vaccine development against dengue has accelerated in recent years with several candidate vaccines currently undergoing clinical trials. In this study we describe the characterization of T cell responses to DENVax, a chimeric dengue-2 PDK-53-based tetravalent vaccine in non-human primates. The vaccine was administered intradermally or subcutaneously with a needle and syringe or a needle-free device (PharmaJet) on day 0 and 60 and the kinetics of CD4$^+$ and CD8$^+$ T cell responses were monitored using flow cytometry. The vaccine was found to elicit T cell responses with both T cell subsets producing proinflammatory cytokines, IFN-γ, TNF-α, and IL-2 one month after priming. CD4$^+$ and CD8$^+$ T cells targeted both structural (E

proteins from each serotype) and non-structural proteins (NS proteins from DENV-2). Following challenge with wtDENV-2 or wtDENV-4, vaccinated animals were protected and frequencies of $CD4^+$ and $CD8^+$ T cells were elevated as compared to the sham immunized animals. These findings highlight the immunogenic profile of DENVax and suggest that dengue-specific T cell responses together with neutralizing antibodies might play a critical role in protection.

610

USEFULNESS OF CLINICAL AND HEMATOLOGIC FINDINGS TO DISCRIMINATE DENGUE FROM OTHER FEBRILE ACUTE ILLNESS IN AN ENDEMIC COUNTRY

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Dengue is the most important arboviral infection in humans, whose frequency has increased in the world over the past 30 years. In tropical countries is essential to differentiate dengue from other infectious diseases. We assembled a cohort of febrile patients recruited from 2003 to 2011, during an epidemic and a non-epidemic dengue period in Colombia. Patients underwent clinical evaluation and hematologic testing within 3 days after the onset of fever. Dengue confirmed cases (DCC) had to have an IgM seroconversion from acute to convalescence samples, a fourfold increase in IgM titers or a positive virologic test (RT-PCR, viral isolation or NS1). Patients not meeting these criteria were referred to as non-dengue cases (NDC). We used multiple logistic regression to determine the contribution of predictors to the likelihood of DCC. We evaluated 1,476 febrile patients (49% DCC, mean age: 22.9 years). Patients with respiratory symptoms - rhinorrhea and cough - were 49% less likely to be DCCs (OR=0.51, 95%CI: 0.40-0.66). Other symptoms such as vomiting (OR=1.43, 95% confidence interval [95%CI]: 1.12-1.81), exanthema (OR=1.52, 95%CI: 1.19-1.94), and clinical findings including somnolence (OR=1.82, 95%CI: 1.30-2.55), body temperature (OR=1.18 per 1°C, 95%CI: 1.05-1.43), and orthostatic hypotension (OR=1.64, 95%CI: 1.13-2.37) increased the probability of DCC as well as a lower platelet count (OR=1.05 per 10,000 platelets/mL reduction, 95%CI: 1.03-1.06). There was an attenuation of the association between leukocyte count and the probability of DCC during the epidemic as compared to the non-epidemic period (OR=1.26 vs.1.44 per 1,000 cells/mL reduction, p=0.036). The model adequately fit the data with an area under de ROC curve of 0.77 (95%CI: 0.75-0.80). The combination of simple clinical findings and easily accessible hematologic tests could help physicians to discriminate dengue from other febrile illnesses in primary health care settings. These findings might be instrumental to developing a diagnosis algorithm in dengue endemic areas.

611

EVALUATION OF THE CLINICAL AND LABORATORY DIAGNOSIS IN PATIENTS WITH SUSPECTED DENGUE

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Dengue fever (DF) is a disease with nonspecific symptoms and a broad clinical spectrum. Laboratory confirmation, using specific serological and virological tests, is essential for a conclusive diagnosis of dengue and to distinguish dengue-like diseases (DLD). Thus, the aim of this study was to evaluate the laboratory data of patients with clinical suspicion of dengue. Patients with acute febrile syndrome were recruited during the epidemic season of 2012 in the State of Ceará, Brazil. Clinical data and blood samples were collected in febrile (< 7 days) and convalescent (≥ 7 days) phases. The following laboratory tests were performed: immunochromatographic NS1 (Bio-Rad®), IgM ELISA (PanBio®), RT-PCR (Qiagen OneStep®) with primers AD3 and AD4, viral isolation (VI) and nonspecific tests (NT). We recruited 88 patients with clinical suspected DF. Nine patients (10%) had a positive NS1 and one (1%) was positive by RT-PCR reaction. Only 44 patients collected convalescent serum samples and 33 of them had a positive IgM test (75%). Of the 50 samples who had VI fulfilled, 3 (6%) were positive for serotype 4 (DEN-4). Thus, 38 patients have been confirmed diagnosis of dengue by at least one of the tests. Regarding symptoms, none analyzed showed significant differences between groups. The most common symptoms for both were: headache, retro-orbital pain, myalgia, arthralgia and prostration. The analysis of NT was significant different only the number of platelets ($p = 0.0086$), being the average of the DF group was 108.000/mm3 and the DLD group 146.000/mm^3. Thus, 48 patients remained without confirmed diagnosis, which may probably mean a failure of diagnostic tools or represent another dengue-like disease. We observed a lower than expected sensibility of virological methods, possibly associated with DEN-4 circulation. Thus, our data suggest the need to improve the accuracy of virological methods to allow the correct diagnosis of dengue and to discover new etiologies of DLD.

612

DENGUE RISK AND PREVENTION IN EPIDEMIC-PRONE RIBEIRAO PRETO, BRAZIL: ANALYSIS OF KNOWLEDGE, ATTITUDES AND PRACTICES

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As many as 100 million cases of dengue occur worldwide each year. Diverse community-level and environmental risk factors for dengue virus transmission include urbanization, population density, climate, personal behaviors, and more. Although dengue risk is both socially and environmentally defined, the role of people's knowledge, attitudes and practices (KAP) concerning disease risk and prevention is not well understood. Accordingly, we studied the KAP of people regarding health-related decisions and behaviors intended to reduce dengue disease in urban Ribeirao Preto, Brazil. A KAP questionnaire was developed with and administered by trained Vector Control Agents of the Municipal Secretariat of Health of Ribeirao Preto in October and November 2012. Adults visiting any of 24 supermarkets in the city were asked to respond to a structured questionnaire. Each supermarket was selected according to vehicular traffic patterns, accessibility, geographic location, and consumer volume to achieve a representative sample. A total of 2,150 adults who consented to participate answered a series of 10 basic sociodemographic questions and 41 open- and closed-ended dengue KAP questions that involved vector ecology, viral transmission, clinical manifestations of the disease, as well as people's attitudes toward disease prevention responsibilities. Results suggest that people possessed adequate knowledge of the disease, especially in terms of recognizing breeding sites, transmission, and basic symptomatology. Dengue was not considered to be a disease of individual-level poverty, rather was thought to be related to neighborhood characteristics. Participants tended to view prevention as a shared responsibility of individuals, neighborhoods, and governmental agencies. Implications of these findings are evaluated in the context of historical patterns of intense transmission in Ribeirao Preto during the past decade.

613

RISK FACTORS FOR DIARRHEA-ASSOCIATED DEATH AMONG CHILDREN IN BOTSWANA IN 2012

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Diarrhea is a leading cause of child mortality in Botswana, a country with high prevalence of childhood malnutrition (~11%) and adult HIV (~25%). For HIV-exposed infants (born to an HIV-infected mother), formula feeding is recommended. A two-fold increase in reported nationwide childhood diarrheal deaths between January-June 2012 prompted an investigation of risk factors to improve prevention and control efforts. A case-control study was conducted at main referral hospitals of 5 districts with the highest diarrhea case-fatality rates. Case-patients (children <5 years who died with gastroenteritis between January 1-June 30, 2012) were compared to age frequency-matched controls (children presenting to surrounding child welfare clinics [CWC]). CWC cards were reviewed for information on malnutrition (weight-for-age z-score <-2), HIV exposure, and exclusive breastfeeding through 6 months of life. Bivariate and multivariate logistic regression were performed to identify risk factors. Sixty-three case-patients and 126 controls were enrolled. Among case-patients, 34 (54%) were male, median age at death was 4 months (range: 0-18 months), 65% had severe dehydration on presentation, and 90% received intravenous fluids. Compared to controls, children who died were more likely to be HIV-exposed (97% versus 34%; adjusted odds ratio [aOR]=32; 95% confidence interval [CI]: 2-530), not exclusively breastfed (94% versus 28%; aOR=13; 95% CI: 2-84), and malnourished (44% versus 2%; aOR=42; 95% CI: 4-427). The vast majority of diarrhea deaths in Botswana occur among HIV-exposed infants who were not exclusively breastfed. Our findings support WHO recommendations to promote breastfeeding regardless of HIV exposure status and for effective treatment of malnutrition as key childhood survival interventions, and could contribute to efforts in Botswana to develop similar policies.

614

BCG NANOEMULSION AS ADJUVANT AND VACCINE DELIVERY SYSTEM

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The development of effective vaccines is essential for controlling disease. Recombinant vaccines are widely used due to its ease to produce and dose adjusts. Unfortunately, recombinant vaccines enjoy low immunogenicity, often requiring the help of adjuvants. Here, we present the development of a novel and potentially effective adjuvant. Inactivated *Mycobacterium bovis* Bacilli Calmette-Guerin (BCG) is used as an active component in the complete Freund adjuvant. Cell wall components (BCG-CWCs) are the most immune-stimulatory part of BCG. Recently, nanotechnology has become integral to medicine and medical research, especially in the areas of drug and vaccine delivery systems. Nanoemulsions (NEs), emulsions with droplet-size in nanometer scale, have been shown to improve the antigenicity of weak antigens as it can easily be up-taken by phagocytes and freely circulate through lymphatic vessels. In addition, NEs can carry antigens to antigen presenting cells (APCs) much more efficiently than larger particles. Given their immune-stimulatory properties, BCG-CWCs in combination with NEs could facilitate antigen recognition by the innate immune system, an essential step for the development of a complete and robust adaptive immune response. Preparation of BCG-CWCs NEs required the isolation and sonication of BCG-CWCs prior to preparation of NEs by homogenization. We were able to determine that the average size of BCG-CWCs NEs is around 300 nm, with a surface potential of -50 mV. A concanavalin A binding assay showed that BCG-CWCs located on the surface of NEs. In an in-vitro monocytes-derived dendritic cells (DCs) assay, BCG-CWCs NEs increased the production of the pro-inflammatory cytokine, IL-12 and induced CD86 expression, a maturation marker of DCs. Immunofluorescent assays showed that BCG-CWCs were up-taken by APCs. The immunogenic properties of BCG-CWCs NEs point to a potentially effective adjuvant and antigen carrier. Further work is now required to measure NE stability and antigen loading efficacy.

615

ESTABLISHMENT OF MOBILE LABORATORIES UP TO RISK GROUP 4 IN COMBINATION WITH CBRN CAPACITY BUILDING IN SUB-SAHARAN AFRICA

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Outbreaks with risk group 3 and 4 pathogens often occur in sub-Saharan Africa. Despite the considerable progress that African countries have made in outbreak management, there is still a need for international assistance in the detection, diagnosis and containment of infectious disease emergencies, such as viral hemorrhagic fevers (VHF). VHF often present with unspecific symptoms of febrile illness, thus making the rapid application of molecular diagnostic techniques for the detection of the pathogens concerned necessary. Therefore, the EuropeAid Cooperation Office of the European Commission has set up this collaborative project, with the overall aim to strengthen scientific cooperation between Europe and Africa in the field of epidemic-prone infectious diseases. Three rapidly deployable laboratory units as well as a collaborative network of European and African institutions will be established in the course of this project. A pool of African and European scientists will be trained in the use of these easy deployable and therefore highly mobile laboratory units, facilitating rapid field based diagnostic response to outbreaks of infectious diseases of risk group 3 and 4 pathogens. One mobile lab each will be stationed in Nigeria, Tanzania and Germany, respectively, and training missions, mock deployments and possibly outbreak response missions in the framework the Global Outbreak Alert and Response Network (GOARN) are planned. The project is linked with WHO, ECDC, and EU public health networks such as ENIVD and QUANDHIP. The European mobile lab consortium consists of partners from the Bernhard-Nocht-Institute, Bundeswehr Institute of Microbiology, Instituto Nazionale per le Malattie Infettive, Irrua-Specialist-Teaching-Hospital, National Institute for Medical Research Dar es Salam, Health Protection Agency, Institute of Virology Marburg, Laboratoire P4 INSERM, and Spiez Laboratory.

616

CONSENT AND ASSENT IN PEDIATRIC RESEARCH IN TROPICAL MEDICINE

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International guidelines suggest that children provide assent for medical research in addition to their parent's consent. However, the concept of assent is confusing and lacks clarity. Assent is traditionally taken to mean "agreement" from someone who is not competent to provide consent. In pediatrics, this means that individuals below the age of majority (usually 18 years old) should provide assent if they are able to. We describe the current international debate surrounding the pediatric consent and assent process and the additional challenges arising when conducting pediatric

research in tropical medicine. These challenges are particularly complicated because diseases are acute, the burden of disease is high, there is a lack of resources and basic infrastructure, there are low levels of education and literacy, and the standard of healthcare is low. In addition, in such contexts, some children make adult-like decisions, are parents themselves or live in complex family situations. In this paper, we argue that the default position should be that competent children should be able to consent for themselves regardless of their age. A competent child who can make his or her own autonomous decisions should be allowed to do so - the required level of competence being relative to a specific decision. This implies, we argue, that the decision about whether to participate in a study, in cases where the decision is of comparable complexity to the decisions the child is used to making in their daily life, should be made by the child. This complexity should not be judged according to first world standards, but in relation to the local setting taking into consideration the benefits, risks and implications of the available choices the child can usually or sometimes make. As in high-income settings, incompetent children should assent in addition to parental consent - assent being involving the child to the extent compatible to his or her maturity and cultural norms, not getting the child's permission to proceed as advocated by current research guidelines. It is important that policies and decisions about particular studies, particular institutions or study sites should be justified, deliberations recorded and final decisions approved by relevant parties.

617

DISEASE ERADICATION: IS AN ECONOMIC PERSPECTIVE USEFUL?

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The global health community pays renewed attention to evaluating the feasibility of elimination and eradication of communicable diseases. Given the intense competition for global health resources in deciding to commit to a national/regional elimination or eradication initiative, economic considerations are important. We developed a framework to show how economics can provide insights on disease eradication and we reviewed the evidence on economic literature. The framework is based on three key questions: 1) Why to eradicate? 2) How to achieve eradication? 3) For Whom? The "Why question" compares costs, health and economic benefits in the long run. The "How question", assesses which intervention/s or strategy/ies should be adopted by which stakeholder; how to generate incentives for each country to eliminate; how much resources would be required; and how these could be mobilized. The "for Whom question", assesses who would benefit from eradication, and the likely impact on equity and fairness. The impact of eradication is of long term. Thus, economic principles are key to assess how much value to give to future health and economic benefits and costs. Elimination and eradication are likely to benefit the most poor with consequences on equity and fairness. As it is not possible to exclude a country/community from the benefits of eradication and every country/community can benefit from it without limiting the others' benefits, disease eradication is a global public good. Economic theory can help assessing the feasibility of eradication by modelling the strategies stakeholders may take and the incentives required to cooperate. Most of the economic literature reviewed is, however, focused on comparing short term costs and consequences. The impact on economic development is rarely explored and issues of equity and fairness are neglected. Our review shows that while economic analyses can be powerful levers to support global eradication policies, methods adopted are based on reductionist approaches failing to consider relevant aspects.

618

COMPARISON OF WRITTEN VERSUS ILLUSTRATED CONSENT METHODS IN A RESOURCE-LIMITED REGION OF THE PERUVIAN AMAZON

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Informed consent is vital to the ethical conduct of research involving humans. Proper informed consent includes a decision-making step by the research subject, following explanation of a study by research personnel knowledgeable in participants' rights. While multiple studies in the U.S. and Europe have compared various informed consent processes, this topic has received little attention in resource-restricted regions such as Peru. Our study aimed to compare comprehension, satisfaction, and length of time of the informed consent process using a text-only document versus an illustrated flip-book. Before enrollment in a febrile surveillance study in the Peruvian Amazon, 254 adults were randomized to receive one of the two consent methods. The two groups were comparable by age, gender, and level of education. Both methods contained identical information and all the study personnel were trained to administer informed consent in a standardized fashion. After subjects finished the consent process, a 17-item questionnaire was administered that measured recall of particular facts about the study and satisfaction with the consent process. We found that those consented with the illustrated method did not have a different level of comprehension than those consented with the written method when we compared the average number of correct answers per subject or the proportion of correct answers for each individual question. Both methods also had similar satisfaction rates and length of consent time (25 minutes for written, 27 minutes for illustrated). Although our results indicate that the two consent processes performed similarly, an illustrated consent form may still offer unmeasured advantages, such as a consistent consent presentation in multi-centric studies and easier comprehension for illiterate study subjects.

619

INCREASING SUPPLY CHAIN KNOWLEDGE, SKILLS AND AVAILABILITY OF TOOLS AMONG HEALTH EXTENSION WORKERS (HEWS) TO IMPROVE COMMUNITY HEALTH SUPPLY CHAIN IN ETHIOPIA

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In Ethiopia, integrated community case management is provided by health extension workers (HEWs) who are trained to provide 16 packages of preventive and curative health services. A baseline assessment identified a lack of basic supply chain management knowledge and skills among HEWs. The SC4CCM Project designed a group training approach, comprising of Integrated Pharmaceutical Logistics System (IPLS) Ready Lessons and Problem Solving (PS) modules, which used existing resources and time by incorporating trainings into routine health center meetings. The intervention aimed to provide affordable, maximum coverage of supply chain management knowledge, skills and tools among HEWs to ensure basic processes and competencies. Training effectiveness was measured using a competency questionnaire, where HEWs were presented with a mock situation and completed a test that assessed components of the IPLS and PS modules. After six months of implementation, the intervention group was characterized by a more rapid pace of IPLS training

rollout to HEWs and greater availability of key supply chain tools including a training manual and blank reporting tools compared to the comparison group. Competency assessments yielded minimal differences across training methods, with HEWs in both groups having difficulty executing complex recording and reporting tasks. Qualitative data from both groups showed that training was not conducted uniformly across regions and that HEWs stated the need for repeated training on more complex topics. Results also showed that specific components of training were found to be effective including problem solving, practical trainings including demonstration, refresher trainings, supportive supervision and regular review meetings. Additionally, minimal differences in competencies could be due to the evaluation being conducted only six months post-training. We therefore concluded that repeated competency testing over time and triangulation of data using other methods such as observation will help supplement and better assess training effectiveness.

620

PAPER-BASED WHOLE CELL BIOSENSORS FOR INEXPENSIVE AND SPECIFIC PHARMACEUTICAL QUALITY TESTING

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Substandard pharmaceuticals have been estimated by the World Health Organization to be 30% of the pharmaceutical market in developing countries. This prevalence of poor quality drugs persists despite an abundance of state of the art analytical methods that exist today. To best address this problem in low resource settings, inexpensive and field-friendly technology is required. Paper-based tests have long been a user-friendly solution to many analytical problems. We have incorporated genetically engineered, whole-cell yeast biosensors into paper, using hydrogels, to make highly specific tests for antibiotics in the tetracycline family. This strain of Saccharomyces cerevisiae are engineered to repress reporter expression in the absence of tetracycline drugs and up-regulate expression in their presence, producing a tightly controlled indicator for this family of antibiotics. Incorporating these yeast into a paper device produces a low-cost, easily transportable test that would have the specificity of a biological system, an improvement over current chemical tests which recognize functional groups, without the need for isolation of antibodies or cell components. Stability testing reveals that these tests remain responsive to analyte for 6 month with refrigeration and can withstand at least 5 days at 47°C. We find that this technology is able to identify the presence of doxycycline at concentrations of 100-3,000 µg/mL. The low-cost nature and portability of this analytical device make it a viable option for use in detecting antibiotics of the tetracycline family in pharmaceuticals in developing countries where the quality of medicines is of concern.

621

POTENTIAL OF NUCLEAR QUADRUPOLE RESONANCE SPECTROSCOPY FOR DETECTION AND CHARACTERIZATION OF COUNTERFEIT MEDICINES

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Counterfeit medicines pose a global and vast growing threat for patients. According to the World Health Organization (WHO), the definition of counterfeit medicines highlights a deliberate and fraudulent mislabelling of products. The development of methods for the identification of medicines is extremely important. Nuclear Quadrupole Resonance (NQR) spectroscopy characterizes solid-state substances containing quadrupolar nuclei (spin quantum number $I > ½$). Since approximately 90% of medicines are in solid form, NQR can detect and identify signals from a broad range of medicines (^{14}N, ^{35}Cl, ^{23}Na etc), focusing only on the active pharmaceutical ingredient (API). This abstract reports the study of detection and characterization of different nitrogenous medicines in the form of tablets, capsules and powder. The aim is to test the NQR response of medicines of different formulations. A quantitative study of classification of a real and suspected counterfeit anti-malarial medicine Metakelfin has also been contacted. A sequence of radio-frequency pulses leads to sample excitation, following the acquisition of the emitted signal. In this study, the "Pulse-spin locking" (PSL) multiple-pulse sequence is used. The linewidth of the NQR signal is described by its characteristic relaxation time constant T_2^* that infers information about the mechanical processing of the medicine. Analgesic Paracetamol (acetaminophen) caplets, capsules and powder of the same brand and quantity, were detected at 2.5637MHz. Variation in the linewidth suggests that NQR infers information about the crystal structure of the sample, specifying the formulation and manufacturing processing of the medicines. The ^{14}N sulfalene component of suspected metakelfin (3.075MHz) tablets is compared with the signal from the genuine batch. The quantitative analysis of the suspected product shows that the sulfanene content is 2.1 times lower than the genuine metakelfin tablets. This study indicates the ability of NQR to detect quantitatively suspected drugs, and the potential of generating drug fingerprints.

622

IMPROVING DATA TO IMPROVE PROGRAMS: IMPLEMENTATION OF A DATA USE PACKAGE AS PART OF THE COMMUNITY CASE MANAGEMENT PROGRAM FOR COMMON CHILDHOOD ILLNESSES IN MALAWI

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The "Implementation Research Embedded in Integrated Community Case Management (CCM) Program: Improving Data to Improve Programs (CCM-IDIP)" Translating Research into Action (TRAction) project is working with CCM programs in Malawi and other countries to improve monitoring, evaluation and use of information. Through a desk review and data quality assessment of the current CCM M&E system, we found a well-defined structure for routine reporting and good levels of reporting and completeness. However data use is low and mostly a top-down approach. Community health workers and their supervisors expressed a keen interest in understanding and using the data that they are collecting and reporting. We worked with district health staff and partners to develop a program to increase data interpretation and use at the community, health center and district levels. By increasing data use at lower levels of the health system, we are hoping to not only improve the overall M&E data quality but allow health workers to quickly make data-based decisions to improve programs. The aim of the package is to improve data use and quality by giving community health workers, health facility and district staff the tools to analyze and interpret the M&E CCM data they routinely report. The package includes (1) general training on data management, use and interpretation; (2) refresher training on the routine reporting forms; (3) simple templates for displaying the monthly CCM implementation strength data; (4) provision of calculators to assist with completing monitoring forms; and (5) working with district staff to identify reporting benchmarks and action thresholds. Since February 2013, two districts in Malawi have been implementing a data use and improvement package. District health staff were trained to provide training on the data use package to the health workers. All CCM-trained workers in the two districts were targeted for training on data use and the package will be implemented for at least three months. A supervisory field mission at the midpoint of implementation show that the templates are being used at all levels and

health worker feedback is on the package is very positive. An endline RDA will be conducted in May 2013 to evaluate whether the program improves data use and quality.

623

A FOURTH DELAY: MALARIA PREVENTION AND NUTRITIONAL SUPPLEMENTATION IN THE FIRST TRIMESTER FOR PREGNANT WOMEN IN BURKINA FASO

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In Burkina Faso, the infant mortality rate is 91.7 per 1,000 live births and the maternal mortality rate is 307.2 per 100,000 live births. Hemorrhage is the direct cause of more than 30% maternal deaths in Burkina Faso each year. Malaria, anemia, HIV/AIDS, and hemoglobinopathies are indirectly responsible for 20% of maternal deaths in Burkina Faso. Literature cites three delays contributing to these maternal deaths namely delays in (1) the decision to seek care, (2) arrival at a health facility and (3) receiving appropriate care upon arrival. This study assessed sociocultural barriers to preventative care for Burkinabé women. From October 2012 to May 2013, focus-group discussions and semi-structured interviews were conducted with pregnant or postpartum women, aged 17 to 40, in one rural and one urban maternity clinic in the medical district of Bogodogo in Burkina Faso. All discussions were recorded, transcribed, analyzed in QSR N-Vivo 10 and then translated into English for the sake of reporting results. Of 60 Women interviewed, 90% cited prenatal visits among the most important steps a pregnant woman should take. However, few women reported attending more than two of the recommended four to six prenatal visits for their current or previous pregnancies and a majority of women believed it was not recommended to have a prenatal visit before the end of the first trimester. Many women claimed that early announcement of pregnancy and prenatal visits would be viewed negatively by other community members and could compromise their health. This delay in prenatal visits translates to a delay in iron and folic-acid supplementation in a population already at risk for anemia in pregnancy and hemorrhage during delivery. None of the women who reported owning insecticide-treated nets claimed to use them every night. The primary reasons women cited for not using mosquito nets were perceived suffocation and oppressive heat. This study suggestions that public awareness campaigns should encourage women to seek early prenatal care to ensure early nutritional support, malaria prophylaxis and counseling in the use of insecticide-treated mosquito nets.

624

MEASURING THE STRENGTH OF COMMUNITY CASE MANAGEMENT IMPLEMENTATION: VALIDATION OF MOBILE PHONE INTERVIEWS WITH COMMUNITY HEALTH WORKERS IN MALAWI

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Since 2008, Health Surveillance Assistants (HSAs) have provided community case management (CCM) of childhood malaria, diarrhea and pneumonia episodes in selected areas of Malawi. In order to gauge the level of program implementation and provide continuous feedback for program improvement, implementers and evaluators require high-quality and inexpensive measures of implementation strength. However the routine CCM monitoring and evaluation system is still being scaled-up in many areas. We have tested and validated a method for collecting implementation strength data at the community level through mobile phone interviews with HSAs. We conducted telephone interviews with 241 CCM-trained HSAs in two districts of Malawi covering training, supervision, utilization and drug stocks. The HSA responses were then validated through direct observation of records at the health centers, drug stocks and CCM registers at the village clinics. A short qualitative module was administered to the HSA at the end of the interview/observation to determine the reasons for any observed discrepancies. We calculated the sensitivity and specificity for key implementation strength indicators based on the cellphone interview using record review/observations as the gold standard. A large proportion (83%) of HSAs were available for mobile phone interview despite recurring network issues. We found sensitivity and specificity for the cell phone interview method to be very high for CCM training status, receipt of initial drug box and provision of services (above 95% for all). Supervision and mentoring indicators were a bit lower although still acceptable (above 80% for all). Current drug stocks and minimum level of key CCM drugs were also high (above 90%). The sensitivity/specificity of the interview method for reported drug stock-outs in the previous three months were a bit lower especially for anti-malarials. We found similar levels of sensitivity and specificity across districts. Many of observed discrepancies were due to HSA errors during the interview such as counting errors, poor understanding of the questions due to network interference and recall mistakes. This study showed that mobile phone interview directly with HSAs provides accurate information on implementation strength, and represents a feasible, low-cost approach of measuring implementation strength indicators in areas where the routine M&E system is still being scaled-up.

625

ENGAGING THE PUBLIC IN PUBLIC HEALTH: THE OPPORTUNITIES AND CHALLENGES OF PARTICIPATORY SURVEILLANCE

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Over the past decade, internet-based participatory surveillance systems for influenza have proven to be fast, accurate, and sensitive. They are also highly scalable, offering the possibility of moving into new areas and targeting more diverse diseases. SaludBoricua.org, a new participatory surveillance system in Puerto Rico, was designed to target multiple acute febrile illnesses - influenza, dengue, and leptospirosis - in an environment where all are present. We are evaluating the ability of self-reported symptoms to differentiate these diseases by comparing participant-generated data to data from traditional, healthcare-based systems and by evaluating data collected in acute febrile illness studies. The system also offers the opportunity to evaluate the impact of interventions, such as vaccination or vector-control, on disease outcomes and characteristics of healthcare system utilization. Moreover, participatory surveillance offers the opportunity to engage the public directly with public health. The public contributes to and has access to the aggregate data and the system forms a direct communication link between public health authorities and the public. Engagement of the public is also one of the largest challenges as these systems must attract users, encourage continued participation, provide useful information, and establish credibility.

626

THE IMPLEMENTATION OF OHASIS, A COMPREHENSIVE HEALTH INFORMATION SYSTEM IN A SOUTH AFRICAN HEALTH LABORATORY SERVICE

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OHASIS is an occupational health information system which gathers data on worker health assessments, hazards and incidents in the workplace. It assists decision makers, health and safety committees and researchers to monitor employee health and safety. OHASIS was developed by the Global Health Research Program (GHRP) at the University of British Columbia (UBC), Canada and installed at the National Health Laboratory Service (NHLS) who embarked on a process of capacity building and empowerment to develop local expertise in the ongoing development thereof. NHLS employs about 7000 employees in 349 pathology laboratories across South Africa and 2 National Institutes. Laboratories range from BSL2 to BSL4. The relationship and co-operation between UBC and the NHLS has been used to implement and strengthen OHASIS in other sites globally. OHASIS has various modules, incident reporting and investigation, workplace assessment and employee health with special reference to HIV and TB. There are complexities in implementation in a resource limited setting and the progress of the system from a paper based to an online system together with the training methods used will be discussed. OHASIS was applied in September 2011 and 413 incidents were reported to date. This is approximately 270 per year, giving an incident rate of 39/1000, an increase compared to the previous 2 years with rates of 33 and 35/1000. Taking incident reporting online should improve reporting further. A survey of health and safety services offered to employees prior to the implementation of OHASIS online has been conducted with 316 respondents showing about 17% required more training with a focus on HIV and TB, 3% indicated that they would never report an incident and 20% conflicted. An e-learning platform is being used to train employees on the online use of OHASIS. Information will be shared on modules being developed like the tracking of hazardous medical waste, facility auditing and equipment maintenance and the interlinking of modules. Introduction of OHASIS in Ghana is being considered.

627

SAVING RAINFOREST WITH A STETHESCOPE: FIVE-YEAR ASSESSMENT OF THE IMPACT OF HEALTH IN HARMONY'S HUMAN AND ENVIRONMENTAL HEALTH INTERVENTIONS IN BORNEO, INDONESIA

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Project ASRI integrates high quality, affordable health care with conservation strategies in Gunung Palung National Park (GPNP) in Borneo, Indonesia. We seek to determine whether the 35,000 patient visits to Klinik ASRI, 1500 mobile clinic visits, ambulance service, community health workers for DOTS TB management, and various livelihood training programs have affected human and environmental health of the region. We conducted a baseline (2007) and follow-up (2012) demographic and KABP survey of 25 villages surrounding the GPNP. Pairs of trained nurses systematically interviewed 1300 households out of 60,000 in the region. Overall health of the population improved. Under 5 mortality was reduced 14.9%; rates of child immunizations increased 25.4%; symptoms of diarrhea, fever, cough < 3 weeks and weight loss were reduced (49.4, 26.2, 59.7, and 68.4% respectively); mosquito net use increased 11.7%; number of births per mother decreased 16.1%; use of birth control increased 12.0%; and, access to water pipes, a restroom and boiling water prior to drinking increased (15.0%, 21.5% and 24.9% respectively). 30 of 32 villages were engaged in ASRI's organic farming, animal husbandry and reforestation programs for livelihood alternatives to illegal logging and there was a 68% decrease in the number of people who illegally logged. ASRI has improved the overall health of the region: ASRI patients were more likely to be healthy and illegal logging is declining.

628

PEACE CORPS VOLUNTEERS SUPPORT INTERACTIVE YOUTH AND COMMUNITY ACTIVITIES FOR WORLD MALARIA DAY - KENYA, 2013

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In 2011, Peace Corps and the President's Malaria Initiative launched Stomping Out Malaria in Africa. In 2012, three Peace Corps Volunteers (PCVs) serving in Kenya received 4 weeks of specialized malaria training to become Malaria Volunteers (MVs). The MVs work with communities, health facilities and the Government of Kenya (GoK) to implement malaria prevention and treatment activities focusing on at-risk and vulnerable populations such as children aged 5-14 years, who have the highest prevalence of malaria in Kenya. The MVs recruited PCVs to implement local World Malaria Day (WMD) activities to increase youth and community awareness of malaria. Participating PCVs attended malaria educational sessions and developed WMD community event plans. Each PCV received a promotional item pack, which included t-shirts, bags and footballs with GoK malaria messaging. Packs were procured and distributed by a partner organization. Limited numbers of socially-marketed insecticide-treated bed nets were provided for resale at community events. Inclusion of community-based organizations, vulnerable populations and GoK staff in the planning process was encouraged. Seventy (61%) of 115 PCVs serving in Kenya planned events in 45 communities across 32 districts. Fifty-eight (87%) PCVs worked in education or health sectors. Sixty-seven (96%) events included school and community football tournaments as part of activities. Fifty-seven schools, 41 community-based organizations, including 10 orphans and vulnerable children's groups, were expected to participate. The participation target was over 8,000 youth, teachers and community members for an average of 120 people per PCV. Football tournaments were held to increase primary and secondary school youth participation in WMD activities. Increasing youth and community awareness of malaria and other health issues by leveraging community-based PCVs to host interactive activities is a strategy that should be more widely adopted.

629

SOLUBLE *PLASMODIUM* APOPTOTIC FACTORS INDUCE APOPTOSIS IN BRAIN VASCULAR AND HEMATOPOIETIC STEM CELLS

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The severity of malaria caused by *Plasmodium falciparum* is determined by many elements, including both parasite apoptotic and host inflammatory factors. One such factor, *P. falciparum* apoptotic factor-1 (PfAF-1), is a soluble factor of parasitized erythrocytes (pRBC) that induces apoptosis in human brain microvascular endothelial cells (HBVEC) and neuroglia. Other factors include submicron vesicles (SV) shed from the plasma membrane of eukaryotic cells and free heme (Hemin) produced during malaria infection. These factors have various effects on vascular endothelia, the blood brain barrier (BBB) and circulating endothelial progenitor cells (cEPC). Serious complications of *P. falciparum* malaria infection are brain endothelial cell damage resulting in BBB dysfunction as well as decreases in endothelial precursors, cEPC, responsible for vascular repair. The independent roles

of these factors in malaria severity are unclear. In addition, Hematopoietic Stem cells (HSC), representative precursors of cEPC, may be susceptible to the apoptotic effects of aforementioned soluble factors, but have not been determined. Our hypothesis of this study was that *P. falciparum* induced apoptotic factors alter cell viability and apoptosis in HBVEC and HSC *in vitro*. HBVEC and HSC viability was assessed using MTT assay and apoptotic indices were measured by activated caspase-3 expression and DNA fragmentation. Significant decreases ($p<0.05$) in viability and increases ($p<0.05$) in apoptosis were observed in PfAF-1, Hemin and SV treated HBVEC and HSC versus non-treated controls. *P. falciparum* apoptotic factors play an important role in inducing apoptosis in HBVEC and HSC. The depletion of cEPC contributes to the serious complication of malaria. *P. falciparum* apoptotic factors could be novel therapeutic targets for severe malaria through decreasing BBB damage and preventing cEPC depletion.

630

POLYMERASE CHAIN REACTION AND HISTOLOGY IN DIAGNOSIS OF PLACENTAL MALARIA IN AN AREA OF UNSTABLE MALARIA TRANSMISSION IN CENTRAL SUDAN

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Prevalence of placental malaria has been widely used as a standard indicator to characterize malaria infection in epidemiologic surveys. Placental malaria poses a greater diagnostic challenge, accurate and sensitive diagnostic tool for malaria infections in pregnancy is needed. A cross sectional study was conducted at Medani Hospital, which serves catchment area which is characterized by unstable malaria transmission. One hundred and seven placentae were investigated for malaria infection using polymerase chain reaction (PCR) and histology. Out of 107 investigated placentae, 33 (30.8%) and 34 (31.8%) were positive for malaria by histology (two (2%) and 31(29.0%) were acute and past infections, respectively) and PCR, respectively. Out of 33 positive by histology, 15 were positive by the PCR while 18 were negative. The sensitivity of the PCR was 45.5% (95% CI: 29.2%- 62.5%). Out of 74 which were negative by histology, 19 were positive by the PCR. This is translated in specificity of 74.3% (95% CI: 63.5%- 83.3%). Of those tested positive by the PCR, 15 were positive by the histology, while 19 were negative. This is translated into a positive predictive value of 44.1% (95% CI: 28.3%- 61.0%). Of those 73 tested negative by the PCR, 55 were negative according to histology while 23 were positive. This is translated into a negative predictive value of 75.3% (95% CI: 64.5%-84.2%). In conclusion, PCR had low sensitivity and specificity in comparison to placental histology, perhaps because the vast majority of the placental infections were past infections. Further research is needed.

631

GENOTYPING OF *PLASMODIUM FALCIPARUM* USING AN AGILENT 2100 BIOANALYZER

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The simultaneous infection of hosts by multiple parasite clones of the highly genetic diverse *Plasmodium falciparum* parasite complicates the understanding of malaria infection. PCR based genotyping of the genes encoding the merozoite surface proteins 1 and 2 (*msp1* and *msp2*) and the glutamate-rich protein (*glurp*) which show high level of polymorphism has been well established to determine the number of parasite clones within one host. Fragment analysis is performed using either gel electrophoresis or capillary electrophoresis (CE). CE has been shown to be highly discriminative compared to gel electrophoresis. This method is however expensive and time consuming. Here we explore the feasibility of using the Agilent 2100 Bioanalyzer, a microfluidic chip-based platform in genotyping *P. falciparum*, and compare the accuracy of the results to capillary electrophoresis in counting the number of clones per infection.

632

INCREASED CEREBROSPINAL FLUID SUPEROXIDE DISMUTASE ACTIVITY IS ASSOCIATED WITH INCREASED SEIZURE ACTIVITY AND PROLONGED DURATION OF COMA IN CEREBRAL MALARIA

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To elucidate the role of oxidative stress in severe malaria pathogenesis and outcomes, plasma Cu/Zn superoxide dismutase (SOD) concentration and activity were assessed in Ugandan children with cerebral malaria (CM, n=172), severe malarial anemia (SMA, n=142), and community children (CC, n=127). In children with CM, plasma and cerebrospinal fluid (CSF) SOD concentration and activity were then compared to mortality and central nervous system (CNS) outcomes. Oxidative stress leads to increased SOD concentration, but SOD activity may reflect ongoing oxidative stress or may be quenched with high levels of ROS. Plasma SOD concentration (median level, ng/ml, [25th, 75th percentile]) was higher in children with CM (322.1, [209.4, 489.4]) than children with SMA (210.6, [138, 320]) (P<0.0001) or CC (194, [138.8-311.4]) (P<0.0001), while levels in CC and SMA did not differ significantly (P=0.6). Plasma SOD activity (mU/ul) was decreased in CM (291, [91.9, 693.5]) and SMA (353, [168, 610.8]) compared to CC (645, [319, 1252.8]) (P<0.0001), but did not differ between CM and SMA (P=0.6). Children with CM who died had an elevated plasma SOD concentration and reduced plasma SOD activity (P=0.05 for both). Among children with CM, those who had seizures after admission had higher CSF SOD concentration (P=0.04) and activity (P=0.006), and CSF SOD activity also correlated positively with number of seizures after admission (Spearman's rho=0.23, P=0.005) and duration of coma (rho=0. 27, P=0.001). CSF SOD activity was inversely correlated with plasma SOD activity (rho=-0.26, P=0.003). Plasma or CSF SOD concentration or activity did not correlate with neurologic deficits at discharge or 6-month follow-up. Plasma SOD concentration is increased and activity decreased in children who die of CM as compared to survivors, but increased CSF SOD activity correlates with increased seizure activity and prolonged coma duration. CNS oxidative stress, as indicated by increased CSF SOD activity, may lead to increased CNS complications in children with CM.

633

SEVERE MALARIA AND MILD MALARIA ARE ASSOCIATED WITH DISTINCT SUBSETS OF *PLASMODIUM FALCIPARUM*

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Malaria can manifest as either severe malaria or mild malaria in children, but the basis for different outcomes is not known. Our epidemiologic studies indicate that high parasite density alone cannot explain severe disease, and we speculated that "severe malaria parasites" have distinct features that increase their virulence over "mild malaria parasites". In microarray and RNA seq analyses, severe malaria parasites from Tanzanian children have distinct signatures. Some of the top differentially regulated genes have undefined roles in the parasite and may be involved in virulence related functions. Gene enrichment analyses coupled with detailed in silico investigation of parasite metabolic pathways indicate that some pathways may be altered in severe malaria parasites, including

enzymes of the purine metabolic pathway and the fatty acid biosynthetic pathway. These pathways may be involved in some aspects of severe disease and their roles are currently under investigation. Unexpectedly, severe malaria parasites also overexpress the variant surface antigen known as VAR2CSA, which was previously shown to be preferentially expressed by pregnancy malaria parasites and involved in adhesion to the placental receptor CSA. Our data suggest that VAR2CSA may have additional roles in disease pathogenesis for pregnant women and children. Differential gene expression in severe and mild malaria parasites has been further confirmed by qPCR analysis in samples isolated from a geographically distant site in Mali. Overall our study indicates that severe and mild malaria parasites display consistent gene expression differences that may be exploited towards therapeutic uses.

634

ANGIOTENSIN II RECEPTORS INHIBIT *PLASMODIUM FALCIPARUM*-INDUCED DISRUPTION OF ENDOTHELIAL CELL JUNCTIONS AND PROTECT MICE AGAINST CEREBRAL MALARIA

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Cerebral malaria (CM) is a complication of severe malaria that leads to the disruption of the blood brain barrier and frequently results in death. We have found that angiotensin II receptors modulate the brain endothelial cell response to *Plasmodium falciparum*-infected erythrocytes by preserving the integrity of interendothelial cell junctions and protecting against experimental CM. We have seen that the disruption of junctions caused by rupture of infected erythrocytes over human brain endothelial cells *in vitro* is inhibited in the presence of blockers of angiotensin II receptor 1 (AT1) or activators of angiotensin II receptor 2 (AT2). Complementary results in the CM mouse model show that treatment of mice with blockers of AT1 or activators of AT2 does not affect levels of *parasitemia*, but result in highly increased survival. Conversely, transgenic mice lacking AT2 receptor present increased susceptibility to CM. These results may facilitate clinical applications, especially since Losartan, an AT1 blocker that showed protective activity *in vitro* and *in vivo*, is commonly used as treatment for hypertension in humans.

635

IMPAIRED RED CELL DEFORMABILITY IN *KNOWLESI* MALARIA IN PROPORTION TO DISEASE SEVERITY

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Plasmodium knowlesi commonly causes severe and fatal malaria in Malaysian Borneo, but little is known about the pathogenesis of disease. In severe *falciparum* malaria, sequestration of parasitized red cells results from cytoadherence to host endothelium and decreased red blood cell deformability (RBC-D), leading to microvascular obstruction, tissue hypoxia and organ dysfunction. In *knowlesi* malaria microvascular accumulation of parasitized cells also occurs, however the mechanisms are unknown. Reduced deformability of *P. knowlesi*-infected red cells has been demonstrated in rhesus macaques, but has not been studied in humans. Using ektacytometry we measured RBC-D in adults with severe (n=21) and non-severe (n=61) *knowlesi* malaria and severe (n=8) and non-severe (n=82) *falciparum* malaria; and 15 healthy controls. At a shear stress of 30 Pascals, RBC-D was reduced in patients with severe (elongation index [EI]=0.496, IQR 0.456-0.528) and non-severe (EI=0.551, IQR 0.494-0.569) *knowlesi* malaria compared to controls (EI=0.583, IQR 0.576-0.590; $p<0.0001$ for both comparisons), and reduced in severe compared to non-severe *knowlesi* malaria ($p=0.002$). RBC-D was similar among patients with severe (EI=0.510, IQR 0.496-0.539) and non-severe *falciparum* malaria (EI=0.516, IQR 0.475-0.558), but was reduced in both groups compared to controls ($p=0.0045$ and $p<0.0001$ respectively). RBC-D did not differ significantly between patients with severe *knowlesi* and severe *falciparum* malaria. Among patients with *knowlesi*, but not *falciparum* malaria, RBC-D was inversely correlated with parasite count (spearman's correlation coefficient =-0.37, $p=0.0006$). Among patients with *knowlesi* malaria, reduced RBC-D may contribute to microvascular sludging, microvascular accumulation of parasitized red cells and impaired organ perfusion in severe disease.

636

A NEW APPROACH TO THE MANAGEMENT OF SEVERE ANEMIA IN *PLASMODIUM FALCIPARUM* INFECTION

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Rupture of invaded red blood cells as they release merozoites into the blood circulation, is a cause of the anaemia in *Plasmodium falciparum* infection. There appears to be yet another and perhaps more serious mechanism that contributes to the severe anaemia seen in *P. falciparum* infection. Some patients on blood transfusion (whole blood or packed cells) for severe anaemia in *P. falciparum* infection have been observed to return to square one (became pale again) within 24 to 72 hours of such transfusion. Giving more blood never changed the situation as they always returned to square one. The issue of jaundice seen in some of these cases tends to start when the spleen began to enlarge and did not correspond to the degree of anaemia as it was usually mild. In some of these patients, there was no jaundice, the level of bilirubin in the blood was normal and there was no urobilirubin in the urine. Perhaps the severe anaemia in *P. falciparum* infection is due to a phenomenon of massive pooling of un-invaded red blood cells from the peripheral circulation into some capillary beds the liver and/or the intestine. This may be an auto-protective mechanism to prevent these cells from being invaded by the *P. falciparum* merocites as they are released into the circulation from the liver. The anaemia in all these cases of severe anaemia that returned to square one after blood transfusion was corrected by adequately treating the malaria and reversing the Auto-Protective massive pooling of un-invaded red blood cells from the peripheral circulation, without further blood transfusion. Perhaps the solution to the management of severe anaemia in *P. falciparum* Infection is not Blood Transfusion but adequate treatment of the malaria fever and the reversal of the auto-protective massive pooling of un-invaded red blood cells from the peripheral circulation phenomenon. The need to investigate the presence of a possible auto-protective massive pooling of un-invaded red blood cells from the peripheral circulation, accounting for the severe anaemia in *P. falciparum* infection can therefore not be over emphasized.

637

COINFECTION OF MURINE GAMMA HERPES VIRUS AND *PLASMODIUM YOELII*: IMPACT ON HOST RESPONSE AND DISEASE SEVERITY

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EBV, a gamma herpes virus and *Plasmodium falciparum* co-infections in early childhood have been associated with the development of endemic Burkitt's lymphoma (eBL), the most common form of pediatric cancer in

equatorial Africa, accounting for nearly 70% of childhood malignancies in these areas. The mutual interaction between viruses and malarial parasites are poorly understood. In order to understand the interaction between gamma herpesvirus and malaria, we established a co-infection model that involves infection of mice with murine gamma -herpesvirus (MHV-68) and *P. yoelii* non-lethal strain (PY17XNL), a murine malaria parasite. Co- infection of MHV68 infected mice with *P. yoelii* results in uncontrolled *parasitemia* and severe anemia (low Hb levels) versus mice singly infected with *P. yoelii*, which readily clears the infection (*parasitemia*) post 3 weeks of infection. Pronounced splenomegaly was observed in co-infected mice accompanied with severe weight loss as compared to singly infected mice. Major alterations in host immune responses and significant perturbations in B and T cell populations were observed. Significantly lower levels of B cells, plasma cells, germinal center cells, (CD4+ and CD8+) T cells were seen in co-infected mice as compared to *P. yoelii* singly infected mice. Our data has demonstrated strong synergy between dual/co infected mice with MHV68 and *P. yoelii*, providing an experimental model in which interference in normal host control of both gamma herpes virus and plasmodium infections are observed. The insights gained from this study may help in understanding the alterations in host responses and gamma herpes virus pathogenesis in co-infected individuals that predispose children to develop Burkitt's lymphoma.

638

PATTERNS OF LEAKAGE FROM RETINAL VESSELS IN PEDIATRIC CEREBRAL MALARIA

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In pediatric cerebral malaria (CM) death and neuro-disability are associated with both retinal vessel leakage and cerebral edema on MRI. We used fluorescein angiography (FA) to investigate presence and severity of leakage from the retinal neurovasculature. We performed FA in the pediatric research ward in Blantyre, Malawi, on children with a clinical definition of CM admitted between 2006-2010. The presence of any leak on admission images was determined by primary grading. Secondary grading assessed detailed leakage subtype and severity on a consecutive set within the series. We report data from the left eye. Leakage was present in 125/170 subjects. In secondary grading (n=87) three main leakage subtypes were found in three mutually exclusive retinal areas (area, % of cases): vessel segment leak (macula 87%, raphe 85%, periphery 89%), focal leak (macula 3%, periphery 15%), punctate multifocal leak (macula 3%, periphery 13%). Each subtype varied in severity, could occur in multiple regions, and could coexist with other subtypes. Vessel segment leak was almost exclusively seen in post-capillary venules and capillaries - only one case had leakage from arterioles. Focal leakage involved areas of 100-500µm in greatest linear diameter and occasionally occurred in large numbers, up to 15. Serial images during admission suggest this is an initial phase of hemorrhage development. Punctate multifocal leak appeared to involve significant dye leakage through very small segments of capillaries, and was often widespread. Unlike focal leak, punctate multifocal leak did not appear to precede hemorrhage. Pediatric CM frequently involves retinal neurovascular leakage, with a wide range of severity. The existence of leakage subtypes suggests that CM may affect the blood-retinal barrier in multiple ways. Insofar as the retinal and brain neurovasculature are similar these subtypes could also reflect brain pathogenesis.

639

ENDOTHELIN-1 TREATMENT INDUCES EXPERIMENTAL CEREBRAL MALARIA DURING *PLASMODIUM BERGHEI* NK65 INFECTION

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Human cerebral malaria (CM) is a life-threatening complication of *Plasmodium falciparum*. Infection of C57BL/6 (B6) mice with *P. berghei* ANKA recapitulates many aspects of CM and is a widely used experimental cerebral malaria (ECM) model. Infection of B6 mice with *P. berghei* NK65 (PbN) does not induce neurological complications. Endothelin-1 (ET-1) is a potent vasoconstrictor with chemotactic properties for leukocytes involved in the pathogenesis of neuroinflammatory diseases. Blockade of the endothelin receptor A prevents the development of ECM suggesting that ET-1 contributes to its pathogenesis. We hypothesized that exogenous treatment of PbN-infected mice with ET-1 triggers the development of ECM. Mice were infected with 106 PbN-parasitized red blood cells and treated with either ET-1 or saline from 4 to 8 days post infection (dpi). PbN-infected mice treated with saline (n=20) did not display ECM and survived until 12 dpi, whereas PbN-infected mice treated with ET-1 (n=18) exhibited signs of ECM such as ataxia and died 4 to 8 dpi. ET-1 treatment had no effect on *parasitemia*, except at 5 dpi when ET-1-treated mice displayed a significant increase in *parasitemia* compared with saline-treated mice. Infected mice had a significant reduction in rectal temperature (RT) and body weight (BW) over the course of infection and the reduction in these parameters was significantly greater in PbN-infected mice treated with ET-1 (>20% weight loss). Uninfected mice treated with ET-1 had a smaller reduction in RT but not in BW. Brain histopathology of PbN-infected mice treated with ET-1 demonstrated the presence of petechial hemorrhages throughout the parenchyma and leukocyte infiltration to the endothelia 6 dpi which were not evident in PbN-infected mice treated with saline or uninfected mice treated with ET-1. These data indicate that ET-1 triggers the development of ECM in PbN-infected mice.

640

POLYAMINE BIOSYNTHESIS ENZYMES ARE CRITICAL FOR THE DEVELOPMENT OF THE MALARIA PARASITE IN THE MAMMALIAN AND MOSQUITO HOSTS

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Polyamines are important organic charged molecules that play important roles in the cell cycle regulation, cell proliferation, senescence and death of eukaryotes and prokaryotes. In addition, polyamine analogues have been considered and applied in cancer therapy. Despite of the constitutive expression of polyamine biosynthesis enzymes during all malaria parasite life cycle stages, little is known about their biosynthesis and cellular functions for *Plasmodium* development in the mosquito and the mammalian hosts. Herein, we applied gene-targeting techniques in *P. yoelii* to target enzymes of this pathway for deletion and for fluorescent tagging, with or without the supplementation of polyamines. Our results indicate that polyamines biosynthesis is critical for the development of life cycle stages of *Plasmodium* in the mammalian and the mosquito hosts. Therefore, our data suggest the potential of polyamine biosynthesis enzymes as multistage drug targets for antimalarial chemotherapy.

641

A POISSON HIERARCHICAL MODELLING APPROACH TO DETECTING COPY NUMBER VARIATION IN THE *PLASMODIUM FALCIPARUM* GENOME USING SEQUENCE COVERAGE DATA

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Next generation sequencing technology has made possible to identify copy number variation (CNV) in a large number of *Plasmodium falciparum* genomes. However, current CNV detection methods rely on statistical assumptions that do not hold in available *P. falciparum* sequencing data, or require fine-tuning the underlying algorithms, a task that may not be feasible when there are a large number of samples under analysis. We propose a new CNV detection methodology based on two Poisson hierarchical models, the Poisson-Gamma and Poisson-Lognormal, with the advantage of being sufficiently flexible to capture different data patterns and with stringency controlled by a statistical parameter similar to the significance level used in traditional statistical analysis. Using 3D7 resequencing coverage data and simulation, our methodology showed a baseline false positive rate in line with the stringency adopted for the analysis. When applied to the non-reference isolate data (HB3, DD2, 7G8, GB4, OX005, OX006), our approach detected known CNV hits, including an amplification of the PfMDR1 locus in DD2 and a large deletion in the CLAG3.2 gene in GB4, and putative novel CNV regions. When compared to the recently available FREEC and cn.MOPS approaches, our findings were more concordant with putative hits from the highest quality array data for the 7G8 and GB4 isolates. These promising results motivate the application of the methodology to a larger collection of *P. falciparum* samples of different origins.

642

THE EVOLUTION AND GENETIC DIVERSITY OF THE CIRCUMSPOROZOITE PROTEIN (CSP) IN *PLASMODIUM SPP.*

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The Circumsporozoite Protein (CSP) is the predominant constituent of the sporozoite surface and plays several fundamental roles, including in the development of the oocyst and the first merozoites in the liver during the pre-erythrocytic cycle. In this investigation, we amplified, cloned and sequenced the CSP gene from several *Plasmodium* species. We separate them into two groups, the Laverania clade that includes *P. falciparum* and related species (5 species) and the *Plasmodium* clade that includes *P. vivax* and related species from monkeys in Africa and Asia (11 species). The alignment and phylogenetic analyses of CSP sequences were performed using only the conserved N and C-terminal regions of the gene. Although the CSP phylogeny follows the trend evidenced by other *loci* (e.g. mtDNA), we found distinctive patterns among *Plasmodium* lineages. From all the species studied, only the two African Cercopithecidae parasites, *P. gonderi* and one from mandrills, lack region I sequence (KLKQP), which plays a critical role in the processing of the CSP in the mammalian host due to its high affinity to heparin sulfate on the surface of liver cells or hepatocytes. We also found that the central tandem repeat region exhibited extensive genetic diversity even among closely related species, or within a single species, in terms of the number of repeats with diverging motifs in many malarial parasites (e.g. P. inui). However, the Laverania clade shows strong conservation in the basic tandem motifs of the CS protein. The asparagine rich motifs, previously reported, PNAN and PNVD, are present not only in *P. falciparum* but also in its closely related species. There are, however, several new motifs found in low frequency such as PNADPN in *P. billcollinsi* and *P. billbrayi* and PNVN in *P. reichenowi*. Finally, we found contrasting patterns of selection acting on the N and C-terminal regions in the two major human parasites. Thus, our findings indicate that the CS protein evolved under different constrains in the *P. falciparum* and *P. vivax* clades.

643

MALARIAL PARASITE DIVERSITY IN CHIMPANZEES: COMPARATIVE APPROACHES TO ASCERTAIN THE EVOLUTION OF *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum, the agent of the most lethal form of human malaria, shares its most recent common ancestor with parasites found in African apes. Here we studied *Plasmodium* lineages found in chimpanzees (*Pan troglodytes*) and explored their recent evolutionary history. We also studied genes orthologous to two currently considered in antimalarial vaccines: merozoite surface protein 2 (MSP2) and the DBL-1 domain from var2CSA gene. In order to study malaria parasite diversity, we screened 74 blood samples of chimpanzees that were collected in the Democratic Republic of Congo in 2010. We amplified, cloned and sequenced the parasites' mitochondrial genomes (mtDNA), the chloroquine resistance transporter (Pfcrt), merozoite protein 2 (MSP2), and the DBL-1 domain from the var2CSA gene, for all positive samples. We found nine positive chimpanzees (12.2%). Four of the nine positive samples were identified as *P. falciparum*, two as *P. reichenowi*-like, one as *P. gaboni*, and two as *P. malariae*. All *P. falciparum* samples were resistant to chloroquine suggesting that they acquired such infections from humans. Time estimates based on this expanded data set support that the evolutionary events leading to *P. falciparum* include an extended period of co-evolution with hominids. Our study indicates that the proposed species: *P. gaboni, P. billrayi*, and *P. billcollinsi* still hold in this extended data set. In the case of msp2, we provide evidence of the recent origin of the two major groups of MSP2 alleles and conclude that they originated after the *P. reichenowi - P. falciparum* split. The var2CSA gene was also found in relatively divergent chimpanzee malaria lineages. This gene accumulated extraordinary genetic polymorphism after the *P. reichenowi* and *P. falciparum* split. These examples support the notion that comparative genomic approaches among *P. falciparum* and its related species will be of great value in understanding the evolution of proteins that are important in parasite invasion of the human red blood cell, as well as those involved in malaria pathogenesis.

644

EVIDENCE OF CLONAL *PLASMODIUM FALCIPARUM* POPULATIONS IN EASTERN PANAMA

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Elimination of malaria from Meso-America urges for the characterization of circulating field strains of *Plasmodium* parasites from the region. In this study we examined 41 DNA samples obtained from *P. falciparum* field isolates collected during an epidemic that occurred in Panama in 2003-2008, and characterized their genetic diversity and relatedness using a SNP-Based Molecular Barcode assay. Principal Component Analysis of the barcode data let us to characterize, map and group the isolates into three distinct clonal sub-populations ($p = 0.0001$), two from the pacific watershed of the isthmus identified as Madugandi and Darien that

clustered together as one clonal group, while the other belonging to the Guna Yala group clustered into two distinct clonal sub-populations. The identical barcodes observed in three subpopulations of *P. falciparum* field isolates indicates that these subpopulations are highly related and could be the result of clonal propagation or epidemic expansion, or both. These findings support the hypothesis that highly related parasite populations are evident in low transmission settings, such as observed in Panama. We anticipate that use of genomic tools such as the molecular barcode will detect changes in malaria parasite population structure that occur as malaria-reducing strategies are implemented regionally to lower transmission. Such tools allow tracking of specific parasite types and we anticipate that these data will help in the planning, design and implementation of malaria elimination programs tailored for the southern region of Meso-America.

645

DRUG INTERACTION EVALUATION OF PYRONARIDINE/ARTESUNATE AND METOPROLOL AND RE-DOSING EVALUATION OF PYRONARIDINE/ARTESUNATE IN HEALTHY VOLUNTEERS

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Pyronaridine/artesunate (PA) is an ACT indicated for treatment of uncomplicated *falciparum* and *vivax* malaria in children and adults. We conducted an open-label randomized study 1) to evaluate for a drug-drug interaction between PA and the CYP2D6 substrate metoprolol, 2) to assess for any effect of PA re-dosing after 60 or 90 days on the pharmacokinetics of pyronaridine, and 3) to explore any relationships between CYP2D6 metabolizer status and pyronaridine pharmacokinetic parameters. Healthy adult subjects were randomized to Arm A or Arm B. Arm A subjects were administered 100 mg metoprolol tartrate alone in the first period, three daily doses of PA with 100 mg metoprolol tartrate with the third PA daily dose in the second period, and three daily doses of PA alone 90 days later in a third period. Arm B subjects received the three day PA regimen alone in the first period, with re-dosing of this three day regimen occurring after 60 days in the second period. Non-compartmental pharmacokinetic parameters were computed for metoprolol, its metabolite alpha-hydroxymetoprolol, and pyronaridine; pyronaridine parameters were based on concentrations obtained during the third day of any given PA dosing period. Pharmacokinetic analysis indicated that co-administration of metoprolol and PA was associated with an average 47.93% increase in metoprolol maximum concentration and a 25.60% increase in metoprolol $AUC_{0\text{-}t}$; these increases most likely resulted from pyronaridine-mediated CYP2D6 inhibition. No interaction effect of metoprolol on pyronaridine was apparent. Furthermore, the pharmacokinetic re-dosing analysis did not suggest a relevant effect of re-dosing after either 60 or 90 days on pyronaridine pharmacokinetics. Finally, a comparative evaluation of pyronaridine pharmacokinetics in poor, intermediate, extensive, and ultra-rapid CYP2D6 metabolizers did not reveal any clear pattern of pharmacokinetic differences.

646

MALARIA THERAPY IN LAGOS, NIGERIA: UPSURGE IN CHLOROQUINE USE COMPARED WITH ACTS

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Artemisinin based combination therapies (ACTs) were adopted in 2005 as standard treatment for uncomplicated malaria in Nigeria to counter resistance of *Plasmodium* to antimalarial drugs such as chloroquine and sulphadoxine/pyrimethamine used previously. Periodic evaluation of the use of ACTs is needed for intervention and control of resistance to this medication. Lagos State in Nigeria contains a potential megacity and a significant proportion of dwellers in the state are treated for malaria in private hospitals. Retrospective studies of 1827 and 1184 prescriptions in 23 private hospitals were done in 2007 and 2012 respectively. Hospitals were selected from 20 local government Areas in Lagos State by multistage sampling method. Use of chloroquine (CQ) constituted 10.1% of anti-malarials prescriptions in 2012 compared with 2.7% in the 2007 study. In contrast, ACTs prescription decreased from 81.1% in 2007 to 53.3% in 2012. Prescriptions which contained only 1 antimalarial agent rose from 10.4% to 30.1% in 2007 and 2012 studies respectively. Triple combinations of antimalarials as ACTs + one antimalarial agent was noted for the first time in the more recent study, these constituted 9.1% of prescriptions. Urgent intervention is needed to promote rational treatment of malaria in Lagos State.

647

WWARN *IN VITRO* PILOT PROJECT: HOW TO REDUCE VARIABILITY FOR *IN VITRO* SUSCEPTIBILITY TESTING OF ANTIMALARIAL DRUGS

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In vitro susceptibility of antimalarial drugs is an important part of resistance surveillance as susceptibility can be assessed without immunity or pharmacokinetic confounders, and the isolated effect of the partner drug in an artemisinin-based combination therapy can be determined. As different methodologies are used between different laboratories, lack of comparability is a challenge for in the interpretation of *in vitro* data. In this pilot project we addressed this issue by assessing intra- and interlaboratory variability and determine factors of variability for *in vitro* testing. Twenty participating laboratories tested the *in vitro* susceptibility of the reference clones 3D7 and W2 to chloroquine, mefloquine, desethylamodiaquine and dihydroartemisinin. Testing was performed with each laboratory's established methodology and WWARN provided the following measures of standardization: 1) genetic validation of the reference clones by microsatellites and pfmdr1 copy number, 2) validated, pre-weighed drugs supplied from the WWARN Reference Material Scheme, and 3) reproducible data analysis using the WWARN *In Vitro* Analysis and Reporting Tool (IVART). In preliminary analysis large interlaboratory variability was demonstrated, especially for dihydroartemisinin. Laboratories using two read-out methods showed low variability between the methods, motivating exploration of the effects of culture conditions as well as read-out methods on variability. Improved understanding of the factors determining variability will be used to make recommendations on strategies to reduce variability to improve comparability and standardization of *in vitro* testing, within and between laboratories. This in turn can result in more reliable and reproducible data and increase usefulness of *in vitro* data for tracking antimalarial drug resistance and validating molecular markers.

648

BENZOXABOROLE ANTIMALARIAL AGENTS: STRUCTURE-ACTIVITY RELATIONSHIP (SAR) OF 6-SUBSTITUTED-1,3-DIHYDRO-1-HYDROXY-2,1-BENZOXABOROLES

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Emerging resistance to the frontline antimalarial drug artemisinin demonstrates the need for new drugs with different structures and targets. To ensure coverage of the widest chemical space, new libraries containing unique chemical scaffolds should be screened. Anacor's library of boron-containing compounds is one such library and has been shown to be a rich source of compounds active against pathogens that cause neglected diseases. For example, SCYX-7158 (AN5568) is in phase I human trials for the treatment of human African trypanosomiasis. Screening of the Anacor boron library has yielded multiple families of benzoxaboroles with activity against malaria parasites. Among these compounds, 6-aryl and 6-aryloxy-1,3-dihydro-1-hydroxy-2,1-benzoxaboroles were identified in screens against cultured malaria parasites, and additional compounds were designed and synthesized. With this process potencies against W2 and 3D7 strain *Plasmodium falciparum* were improved 2500-fold (IC_{50} 1.0 μM to 0.4 nM). Based on available data, structure-activity relationships show that the substituent at the benzoxaborole 7-position has significant impact on antimalarial potency. The results of a more detailed SAR investigation of these benzoxaborole compounds will be presented.

649

A RANDOMIZED STUDY TO ASSESS THE EFFICACY AND TOLERABILITY OF THREE ARTEMISININ BASED COMBINATION THERAPIES FOR THE TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA IN THE DEMOCRATIC REPUBLIC OF CONGO

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With increasing resistance in Africa to a range of antimalarial drugs, new treatment options for *Plasmodium falciparum* malaria are urgently needed. We tested safety, tolerability and efficacy of the artemisinin-based combination treatment dihydroartemisinin-piperaquine (DP) in children with uncomplicated *P. falciparum* malaria in the Democratic Republic of Congo (DRC). We compared DP to amodiaquine-artesunate (AS), first-line treatment in DRC since 2006 and artemether-lumefantrine (AL) recently added to the first-line.An open label, individually randomized, controlled trial was carried out in 2011-12 in a malaria endemic sector of Kinshasa. Children aged 3 to 59 months with uncomplicated *P. falciparum* malaria were randomly allocated to AS, AL or DP. Children were hospitalized for three days, given supervised treatment and followed-up weekly for 42 days. The primary endpoint was efficacy defined as the *P. falciparum* PCR-adjusted cure rate assessed by day 42.Six hundred and eighty four patients were recruited. The median *parasitemia* on admission was 32,000 parasites/μL (range 2,040 to 199,960) and 6% of patients had >175,000 parasites/μL. The mean time for the log *parasitemia* to decrease by 50% was 2.69 hours (SD 0.84; range 2.56 to7.29) and similar between arms (p=0.18). All patients cleared the infection completely within 72 hours of admission. The PCR unadjusted cure rates by day 42 were AS=73.5%, AL=70.6% and DPQ=86.8% (p=0.001). Early treatment failure occurred in three patients (0.5%), one in each arm. The PCR adjusted cure rates were AS=93.8% AL=93.1%, DP=94.8% (p=0.76). The mean PCV at admission was 30.2% (SD 4.9) and an overall mean reduction of 10.1% (SD 8.3) was observed with 6.0% of patients experiencing a reduction of >25% of the admission value, with no difference between arms (p=0.65). Ten patients required a blood transfusion during hospitalization. The regimens were well tolerated and there were no drug-related serious adverse event related to the treatments. All combinations were equally effective in treating the disease with a favourable safety and tolerability profile. DP provided greater protection from new episodes of malaria during the 42 days follow-up compared to either AL or AS (Trial Registration ISRCTN20984426).

650

EVALUATION OF THE QUALITY OF MALARIA TREATMENT AND OF MALARIA IN PREGNANCY IN HEALTH FACILITIES IN MALI

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In Mali, malaria is the major cause of morbidity and mortality especially for children under 5 and pregnant women. Since 2007 Mali has benefited from the President's Malaria Initiative (PMI) funding. In order to assess the impact of this support a cross-sectional study to evaluate malaria case management and antenatal care in health facilities was undertaken. Three methods of gathering information were used: 1) Provider observations during curative or antenatal care, 2) Re-examination of the patients whose consultation (curative and ANC) was previously observed, 3) Interviews of providers, patients and health facility managers. ACT were available for free for children under five/pregnant women or for sale for other ages in all of our sample of health facilities, the vast majority of health facilities did not have mosquito nets for distribution. 57.8% of providers reported having participated in a course of formal training on malaria case management using ACTs. For uncomplicated malaria in children under 5, 79.3% of diagnoses were without diagnostic error. However, 52.6% of patients with suspected malaria were not given a diagnostic test. All malaria prescriptions were analyzed. For uncomplicated malaria in children under 5, the correct information for the number of days was given 12 of 13 times, every time for the number of doses per day, and 11 of 13 times for the name of the drug and definition of the dose. For severe case management in children under 5, 6 out of 10 providers gave the correct number of treatments per day, and 2 out of 10 prescribed the correct number of days. The results are even poorer for patients over five years: 11 of 12 prescriptions gave a wrong number of days of treatment and 6 the incorrect number of doses per day. In conclusion, management of uncomplicated malaria is satisfactory, but the management of severe malaria is weak. National treatment guidelines requiring laboratory confirmation of all malaria cases are not followed in half of the cases, confirmation is particularly low for suspected cases in pregnant women.

651

CLINICAL DETERMINANTS FOR EARLY PARASITOLOGICAL RESPONSE IN PATIENTS DIAGNOSED WITH UNCOMPLICATED MALARIA IN AFRICA TREATED WITH ARTEMISININ COMBINATION THERAPIES: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Slow clinical and parasitological response after artemisinin therapy for uncomplicated *Plasmodium falciparum* malaria has been reported in the Mekong region. Further spread of these parasites poses a major global public health threat, especially in sub-Saharan Africa where the

disease burden is greatest. In this pooled analysis, the geographical and temporal trends of early parasitological response following artemisinin-based combination therapy (ACT) treatment in African clinical trials. The cofactors affecting early and late treatment outcome were investigated. Individual patient data from efficacy trials were shared with the Worldwide Antimalarial Resistance Network (WWARN) and pooled using a standardised methodology. Data from 52 clinical efficacy studies (N=19,078) conducted in Africa (2002-2011) of Artemether-Lumefantrine (AL, n=9,377), Artesunate-Amodiaquine (ASAQ, n=6,167) and Dihydroartemisinin-Piperaquine (DP, n=3,534) were included in the analyses. The risk of remaining parasitaemic increased on day 1 [AOR: 1.5, 95% CI: 1.3-1.7, P<0.001], but decreased on day 2 [AOR: 0.85, 95% CI: 0.74-0.98, P=0.02] and day 3 [AOR: 0.7, 95% CI: 0.5-0.9, P=0.01], reflecting probably a reduction in population-level clinical immunity. Baseline parasitaemia at enrolment was the most important risk factor affecting parasitological response at day 1 [AOR: 1.75, 95% CI: 1.7-1.8, P<0.001], day 2 [AOR: 1.4, 95% CI: 1.3-1.5, P<0.001] and day 3 [AOR: 1.3, 95% CI: 1.1-1.5, P<0.01]. Patients who remained parasitaemic on any of the first three days were at substantially greater risk of subsequent recrudescent infection: AHR=1.6 [95% CI: 1.1-2.2, p=0.01] on day 1, 1.6 [95% CI: 1.1-2.4, p=0.02] on day 2 and 3.5 [95% CI: 1.7-7.1, p<0.001] on day 3. Although a delay in early parasite clearance was associated with treatment failure, there was no evidence from the data examined that the overall speed of clearance is declining in Africa. Drug resistance surveillance needs to be vigilant and strengthened to limit geographical and temporal gaps in data.

652

TREATMENT OF SEVERE MALARIA - AN OPERATIONAL COMPARISON BETWEEN QUININE AND ARTESUNATE FOR THE TREATMENT OF SEVERE MALARIA IN SEVEN HEALTH FACILITIES IN THE DEMOCRATIC REPUBLIC OF THE CONGO - "MATIAS"

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About 8 million cases of severe malaria occur each year, with a particularly heavy toll on pregnant women and children. The Democratic Republic of the Congo (DRC) is the second most malarious country in the world (after Nigeria) and has the highest severe malaria burden. Recent trials comparing injectable artesunate with quinine have demonstrated relative mortality reductions of 34.7% in adults and 22.5% in children, with lower side effects. In 2011, WHO recommended injectable artesunate as the preferred option for treatment of severe malaria. In early 2012 the National Malaria Control Program (PNLP) of the DRC adopted the WHO guidelines and changed the policy for treatment of severe malaria in children and adults to injectable artesunate. However, the nationwide rollout is a complex undertaking, requiring many operational and clinical adaptations. To provide information and support this process in a country marred by technical and logistical challenges, a limited scope implementation study was designed. The study comprises four key components: a) clinical safety and efficacy assessment on the basis of routine patient information; b) time-and-motion study to study operational parameters; c) feasibility and acceptability assessments through provider and patient/caretaker questionnaires; d) financial cost analysis. The study is conducted in seven health facilities in three rural and one urban health zones of the DRC. In a first phase 410 patients were treated with injectable quinine. After a transition and training phase 350 patients will be enrolled until the end of June 2013 and treated with injectable artesunate. Following recent reports on hemolytic anemia being potentially related to the use of injectable artesunate and CDC's January 2013 recommendation for an expanded follow-up phase of patients of 28 days, the study was amended accordingly. Hemoglobin levels are now measured at days 7, 14, 21 and 28 after treatment. The overall fatality rate in the population treated with quinine was 3.4%; the results for injectable artesunate are expected for September 2013.

653

IMPACT OF MULTIPLE DOSES OF INTERMITTENT PREVENTIVE TREATMENT REGIMENS IN PREGNANT WOMEN ON BIRTH WEIGHT IN SOUTHERN PROVINCE, ZAMBIA

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Malaria in pregnancy (MiP) causes negative neonatal outcomes, such as low birth weight (LBW), a significant contributor to infant mortality. The Zambian Ministry of Health recommends 3 doses of sulfadoxine-pyrimethamine (SP) as intermittent preventive therapy in pregnancy (IPTp) to prevent MiP. The new World Health Organization (WHO) guidelines now recommend monthly SP IPTp as a replacement for the previously recommended 2-dose regimen, after a meta-analysis demonstrated the superiority of 3 or more doses in preventing LBW and other complications of MiP. This analysis compares the impact of the current IPTp-SP guidelines (3 doses or more) to the prior guidelines (2 doses or more) in reducing LBW in Southern Province, Zambia. We hypothesized that the 3-dose group will have a lower percentage of LBW newborns and a higher mean birth weight. We performed a secondary analysis using a subset of participants (n=14,414) enrolled in the Zambia Chlorhexidine Application Trial (ZamCAT) in Southern Province, Zambia. Using ANOVA and multivariate linear regressions, we assessed the impact of the number of SP doses (0, 1,2,3,4+) on birth weight. Logistic regression models were used to compare the proportion of LBW infants between groups, and to examine the impact of the previous SP IPTp guidelines (2 doses versus no SP) compared to the current guidelines (3+ doses versus no SP) on LBW. We found an increase in birth weight and a decrease in the proportion of LBW infants with increasing number of SP doses (p <0.0001). Treatment with the prior WHO guidelines provides a trend towards a protective effect against LBW among premature newborns (OR=0.77, 95% CI 0.58, 1.03) but not among newborns of normal gestational age (OR= 1.15, 95%CI 0.9,1.46). A stronger protective effect was observed with the new guidelines, particularly among premature newborns (OR=0.64, 95%CI 0.50, 0.80). The new IPTp recommendations may help reduce the risk of LBW among premature newborns, and thus may serve to decrease their risk of early mortality even in the context of high levels of SP resistance.

654

ASSESSING THE EFFECT OF THE RECOMMENDED ARTEMETHER-LUMEFANTRINE DOSING REGIMEN ON THE RISK OF TREATMENT FAILURE IN PATIENTS DIAGNOSED WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Artemether-lumefantrine (AL) is the first line antimalarial treatment in 49 countries, administered according to four weight bands. Patients at the margins of these bands can receive significant deviation from the target dose. To assess the impact of weight adjusted (mg/kg) dose variations in therapeutic efficacy, individual patient data were shared with the WorldWide Antimalarial Resistance Network (WWARN) and collated using standardised methodology. Risk factors associated with recrudescence were evaluated using Cox's regression model with shared frailty on study sites. Data from 14,986 patients (65 efficacy studies between 1996 and 2011) with uncomplicated *Plasmodium falciparum* malaria were included in the analyses (Africa: 12,179, Asia: 2,648, South America: 159). A total of 320 Polymerase Chain Reaction (PCR)-confirmed recrudescent infections were reported. The median (IQR) total lumefantrine dose was 68.6 mg/kg (57.6-80.0), with children <1 year receiving the greatest dose [87.8 mg/kg (75.6-98.6)], compared to those between 1 and 5 years [67.9 mg/kg (59.0-80.0)], 5 and 12 years [74.5 mg/kg (65.5-83.1)] and ≥12 years [57.6 mg/kg (49.9-65.5)]. The median (IQR) mg/kg dose of lumefantrine in patients failing treatment was 70.6 mg/kg (57.6-80.0), and did not differ significantly from those who were cured [68.6 mg/kg (57.6-80)]. In a multivariate model the log of the baseline parasitaemia and young age (1 to 5 years) were both significant risk factors for recrudescent infections (AHR=1.14 [95 CI%: 1.04-1.24, p=0.003] and 2.27 [95 % CI: 1.43-3.58, p<0.001]) respectively, accounting for 41% of all treatment failures. Treatment supervision and co-administration without a fatty meal were not associated with increased risk of recrudescence. In this pooled clinical analysis, the mg/kg dose of lumefantrine administered was not correlated with treatment failure suggesting that current dosing strategies of AL are robust.

655

SEASONAL MALARIA CHEMOPREVENTION IN SENEGAL: FROM RESEARCH TO POLICY

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Seasonal malaria chemoprevention (SMC) is a new strategy for malaria control in children. Studies conducted in Sahelian and sub-Sahelian Africa have shown that SMC is highly effective, safe and can be delivered at large scale with high coverage in areas where malaria is seasonal. In 2011, through a cluster randomized trial in southern Senegal, we investigated the effectiveness of SMC combined with community case management for preventing the burden of malaria in the study area. Other objectives of the project included an assessment of the feasibility and tolerability of SMC in older siblings (5-9 years old) and for a period of 5 months. The primary endpoint was the incidence of malaria (fever or history of fever with a positive RDT). Preliminary results show a 82 % protective efficacy of SMC. A positive impact on severe anemia and *parasitemia* has also been found. SMC is now adopted as national policy in Senegal and will be implemented as of August this year in 4 regions (Tambacounda, Kédougou, Kolda, Velingara) all located in the southern part of the country totaling around 550000 children under 10 years of age. In these regions, the clinical attack rate of malaria is greater than 0.1 attack per transmission season in children under 10 years of age. The impact of SMC on malaria morbidity and mortality will be evaluated and effects on natural acquisition of immunity explored using a case control approach. Prevalence of molecular markers of resistance to SMC drugs will be measured in blood samples in used RDTs. In the west African sub region 9 countries with Senegal have started the process to incorporate SMC among their malaria control strategies. A partnership between LSHTM, UCAD, WHO and WARN has been set up to help these countries to elaborate their implementation plan.

656

HEMOGLOBIN LOSS AND ITS ASSOCIATION WITH PROTECTION AT RELATIVELY LOW PARASITEMIA IS INFLUENCED BY A HOST GENETIC FACTOR IN SEMI-IMMUNE MICE INFECTED WITH *PLASMODIUM BERGHEI* ANKA

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Human studies and our previous animal studies have shown that there are individual responses towards malarial infection even under the same malarial transmission intensity. These studies also show that individuals with low haemoglobin (Hb) are protected. We, therefore hypothesize that host genetic factors is/are influencing these differences affecting the extent to which Hb is synthesized during malaria infection. In testing this hypothesis we crossed two mice strains (Balb/c of low parasitaemia) and CBA (moderately high parasitaemic) to get the progeny, called the F1. Balb/c (8), CBA (8) and F1 (12) were taken through 6-7 cycles of infection (with *Plasmodium berghei* ANKA) and treatment (with chloroquine/pyremethamine) to generate semi-immune status. Parasitaemia and haematological parameters were monitored. Kinetics of antibody production, cytokine levels (in serum and cultured supernatant of stimulated spleen cells) and CD4+CD25+T regulatory cells were evaluated by ELISA, bead-based multiplex assay kit and FACs respectively; at days 0, 16, 28 for Balb/c and F1, and days 0, 8 and 12 for CBA. Similar survival (>70%), mean %Hb loss (45%) and mean parasitaemia (5%) was observed in Balb/c and F1, while 0% survival, mean %Hb loss (80%) and mean parasitaemia of 15% was observed in CBA. IgG subtypes were two times higher in Balb/c and F1 than CBA. While IL1a, IL4, IL10, IL12a, IFNγ and TNFα were similar in the three mice strains, IL17 was 4.5 times higher in Balb/c and F1 than CBA. Increasing trend of cytokines levels was observed in CBA whilst a maximum cytokine level was observed at D16 (point at which recovery from parasitaemia occurs, with lowest Hb) in the Balb/c and F1. CD4+CD25+ Treg cells in CBA were similar on days measured, but lower than those of Balb/c and F1. In conclusion, innate mechanism of Hb loss in controlling parasitaemia level, hence survival is similar in Balb/c and F1. A genetic factor controlling this Hb loss in Balb/c is passed onto the F1 progeny.

657

HEMOGLOBIN-α 2-PROMOTER VARIATION INFLUENCES SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM*-ASSOCIATED ANEMIA

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Severe malarial anemia [SMA, hemoglobin (Hb) less than 5.0g/dL], due to infection with *Plasmodium falciparum*, is a leading cause of morbidity and mortality in African children. The underlying genotypic traits that influence SMA have not been fully elucidated. Current findings using

high-throughput genotyping [Human BeadChip' (2.45x106 markers)] and global gene expression arrays [HumanHT-12 v4 BeadChip (47,231 probes)] to investigate a pediatric population in Kenya (3-36mos) identified a significant association between several genetic variants and SMA. Whole genome genotyping in a subset (n=100) of children with *falciparum* malaria (non-SMA vs. SMA) revealed >2 copies of hemoglobin-α2 (HBA2) and several polymorphic variants in the promoter region of children with SMA. In addition, global gene expression profiling showed a 1.81-fold change in HBA2 in the SMA group. HBA2 codes for one of the two α-chains of hemoglobin. Previous studies demonstrated that similar polymorphisms generate hemoglobinopathies that protect against *P. vivax* and *P. falciparum* infections in pediatric cohorts. To extend and confirm the whole genome findings, we performed in silico analysis for HBA2 and identified two (potentially) functional SNPs (rs1203833 and rs2974771) that were then genotyped in a larger cohort (n=739), followed by construction of haplotypes. Binary logistic regression analysis, controlling for covariates (age, gender, G6PD, HIV-1, *bacteremia*, α-thalassemia, and HbAS status) revealed that carriage of the TG haplotype (-1789T/-4314G) increased susceptibility to SMA (OR: 1.61, 95%CI: 1.11-2.32, P<0.05). In addition, a reduced erythropoietic response (RPI<2) was observed in carriers of the AG (-4314A/G; OR: 0.65, 95%CI: 0.44-0.96, P<0.05) and TT (-1789C/T; OR: 0.50, 95%CI: 0.29-0.85, P<0.05) genotypes, while carriage of the CA (-1789C/-4314A) haplotype was associated with enhanced erythroid production (OR: 3.11, 95%CI: 1.34-7.22, P<0.001). Taken together, these results demonstrate that variation in HBA2 is associated with susceptibility to SMA and altered erythropoietic responses.

658

A DELETION OF 3.7 KILOBASES OF DNA IN THE ALPHA GLOBIN GENE (-α3.7 THALASSEMIA) PROTECTS CHILDREN AGAINST SEVERE MALARIAL ANEMIA IN WESTERN KENYA

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Alpha (α)+ thalassemias (α-thal) result from deletions of one or both of the duplicated α-globin genes and/or inactivating mutations. In sub-Saharan Africa, the most common deletions are the -α3.7, often presenting as heterozygous (-α3.7/αα) and homozygous (-α3.7/-α3.7) forms. Although both heterozygous and homozygous α+ thals confer protection against fatal *Plasmodium falciparum*malaria, their effect on severe malarial anemia (SMA; Hb less than 5.0g/dL; with any density *parasitemia*) as the primary clinical outcome of severe disease has not been determined. As such, children (less than 3 months; n=990) living in a holoendemic malaria transmission region of western Kenya were recruited into the study and grouped into three categories: aparasitemic controls (AC; n=239), non-SMA (Hb less than 5.0g/dL; n=611), and SMA (Hb less than 5.0g/dL; n=140). a3.7 thal deletion variants were genotyped and their effect(s) on clinical outcomes was investigated. The proportion of deletion variants .distributed across the groups was: aa/aa, 0.221 (AC), 0.622 (non-SMA), and 0.156 (SMA); -a3.7/aa, 0.242(AC), 0.636 (non-SMA), and 0.121 (SMA); and a3.7/-a3.7, 0.277 (AC), 0.580 (non-SMA), and 0.143 (SMA). The distribution frequency across the clinical groups were comparable (P=0.370). Multinomial logistic regression analysis modeling, controlling for confounders, indicated that homozygous carrier of the -a3.7/-a3.7 variants were significantly protected against both non-SMA [OR=0.615 (95%CI 0.405-0.935) P=0.023] and SMA [OR=0.339 (95%CI 0.138-0.834); P=0.019]. In addition, carriage of the -a/aa genotypes partially protected against non-SMA [OR=0.807 (95%CI 0.553-1.073); P=0.065] and SMA [OR=0.566 (95%CI 0.242-1.171); P=0.091]. Taken together, these results demonstrate that α+-thal deletions confer a protective advantage against malarial anemia in regions of holoendemic malaria transmission.

659

POTENTIAL *PLASMODIUM* SPECIES-SPECIFIC VULNERABILITIES TO TRANSMISSION BLOCKING DUE TO HOST IMMUNE RESPONSES: INSIGHTS FROM MATHEMATICAL MODELING

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Experiments involving transmission of avian and rodent malarias show that host factors can attack the gamete forms of the parasites in the midgut of mosquito vectors, an effect known as transmission blocking. In addition, some human patients infected with either *Plasmodium falciparum* or *P. vivax* develop antibodies against the gamete forms of those species. However, a 1977 field study in Gambia suggested that host immune responses directed against the within-host gametocyte forms (precursors of the gametes, before uptake by mosquitoes) had little effect on *P. falciparum* transmission. On the other hand, a 2008 field study (again in Gambia) showed that patients who had antibodies to surface antigens of transmissible *P. falciparum* gametocytes cleared gametocytes earlier than those who did not. Using theory from population biology, we developed mathematical models of the within-host asexual forms and gametocytes interacting with host innate and antibody immune responses. Models were tailored for the specific life cycles of *P. falciparum* and *P. vivax*, and for wide ranges in host immune capacity to detect and clear parasites. We show that for both *Plasmodium* species, for the same ability to detect and clear a targeted parasite stage, antibodies to the immature (pre-transmissible) gametocytes would be more effective in reducing the density of transmissible gametocytes than antibodies directly targeting the transmissible forms. But we also found that due to the longer time needed for gametocytes of *P. falciparum* to mature, this species is much more vulnerable than *P. vivax* to transmission blocking by host antibodies to the immature gametocytes. Since field studies indicate that transmissible gametocyte density in malaria patients infected with either species are the same, on average, our results suggest that *P. falciparum* has evolved mechanisms to evade or suppress host immune responses that can effectively eliminate immature gametocytes.

660

CYTOKINES AND ANTIBODIES LEVELS AND PREVALENCE OF CONGENITAL AND NEONATAL MALARIA IN MALI

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At the Pediatric ward of the National Teaching Hospital Gabriel Toure, which is the main tertiary Pediatric reference hospital of the country, neonatal mortality was 30%, 36.7% and 61% in 1997, 1999 and 2000, respectively. Because of scarce resources, no exact etiology of these deaths is known. However, based on clinical signs, the great majority of these illnesses are categorized as of infectious origin. Pediatricians use their clinical judgment to prescribe antibiotics and other treatments without laboratory evidence of etiology. We proposed to test the hypothesis that the prevalence of congenital and/or acquired malaria is negligible in new born infants in Mali. We used sensitive molecular biology and biochemical methods to measure the prevalence of malaria in preterm infants and in neonates admitted to the Pediatric ward of Hospital Gabriel Toure. We found that all 300 infants were negative for malaria *parasitemia* using both microscopy and PCR. The OptiMal IT was positive for *P. falciparum* in 3 infants (1%). Among the 146 mothers included in the study we found that 0 (0%), 1 (0.7%) and 10 (6.8%) were positive for malaria parasites using microscopy, OptiMal IT and PCR, respectively. Cytokine analyses showed that neonates had a strong anti-inflammatory response, significantly higher than their mothers (p< 0.05). The response was significantly higher than that of PCR (+) mothers for IL2 and IFN-gamma. Similarly, PCR (-) mothers had higher levels of MSP3 and GLURP

antibodies. Our data suggest that there is no malaria in congenital and neonate population. Immunity factors may play a key role in the protection of congenital and neonate infants.

661

DOES LIGAND BINDING ALTER THE IMMUNOGENICITY OF *PLASMODIUM FALCIPARUM* AMA1?

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Apical membrane antigen 1 (AMA1) has been considered a leading malaria vaccine candidate but polymorphisms in AMA1 limit its efficacy. In a recent Phase 2 trial in Mali an AMA1 vaccine had significant efficacy only against parasites expressing a form of AMA1 related to that in the vaccine1. Combining multiple allelic forms of AMA1 in a vaccine may overcome this problem by directing antibody responses to conserved epitopes but this would increase the cost of an AMA1 vaccine. A possible alternative approach was suggested by the observations that the most polymorphic region (C1-L) on the surface of AMA1 is adjacent to the RON2 binding site on AMA1, and when a ligand binds into this hydrophobic pocket, there is a significant rearrangement of the conserved domain II loop. Groups of mice were immunized with three different AMA1-peptide complexes; two peptides (R1 and R3) were isolated from a phage-displayed peptide library and the third (RON2L) is a segment of the natural ligand of AMA1. Sera from the mice were analysed by direct and inhibition ELISAs using a variety of forms of AMA1, including chimeric constructs displaying regions of *P. falciparum* AMA1 on a *P. berghei* AMA1 background. Although the complexes induced good responses there was little evidence that the antibodies were more cross-reactive than those induced by uncomplexed AMA1. The results of this preliminary experiment are not encouraging but we will explore this approach further using immunogens in which the peptide ligands have been chemically cross-linked to AMA1.

662

ANTIBODIES THAT PROMOTE PHAGOCYTOSIS OF *PLASMODIUM FALCIPARUM* MEROZOITES ARE ASSOCIATED WITH PROTECTION AGAINST MALARIA AND CAN BE INDUCED BY HUMAN VACCINATION

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Malaria illness develops during the blood-stage infection when the merozoites form of the parasite infect human erythrocytes and replicate inside them. Merozoite antigens are important targets of antibodies that are thought to be the key mediators of protective immunity, and merozoite antigens have long been regarded as promising vaccine candidates. The protective mechanisms of antibodies against merozoite antigens are not well understood, but may include opsonization of merozoites for phagocytic clearance. Currently, there is a lack of assays to measure functional antibody activity in studies of acquired and vaccine-induced immunity in humans. To address these questions, we developed a high-throughput assay to quantify antibody-mediated phagocytosis using a human monocyte cell line and purified *P. falciparum* merozoites. We used this assay to assess the opsonic activity of antibodies in cohort studies of children and adults in Kenya, and a phase 1 vaccine trial of a major merozoite surface antigen. We found that antibodies that promote opsonic phagocytosis were acquired with increasing age and exposure to malaria, and broadly correlated with IgG levels to merozoite antigens measured by ELISA. Importantly, high levels of opsonic phagocytosis activity among children were prospectively associated with a decreased risk of malaria, suggesting a role in protection. Human immunization with recombinant merozoite surface protein 2 (MSP2), an abundant protein on the merozoite surface, generated cytophilic antibodies, IgG1 and IgG3, that bound the merozoite surface and promoted opsonic phagocytosis. Our findings suggest that opsonic phagocytosis of merozoites may be an important mechanism by which antibodies to merozoite antigens contribute to protective immunity to malaria and and support the further development of merozoite surface proteins as potential malaria vaccine antigens. Furthermore, these studies have established a high-throughput assay to measure the functional activity of antibodies to merozoites to investigate acquired and vaccine-induced human immunity.

663

IMMUNOLOGICAL CORRELATES OF REINFECTION IMMUNITY IN MURINE MALARIA *PLASMODIUM YOELII*

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Immunological mediators of reinfection immunity in malaria are poorly understood. We studied the immune correlates of immunity during reinfection with non-lethal *Plasmodium yoelii* 17XNL parasites in BALB/c mice that had once cleared their infection with the homologous parasite. Absence of *parasitemia* in BALB/c mice reinfected three and five months after primary parasite clearance indicated the presence of solid protective immunity in these mice. Although comparable levels of $CD19^+$ mature B cells were observed in infection cleared, post-challenged and age matched control mice after 5 months of parasite clearance, frequencies of IgG1 isotype switched memory B cells and $CD19^+CD138^+$ plasmablasts were significantly higher in post challenged mice as compared to infection cleared and age matched, malaria naive controls ($P<0.0001$) suggesting a protective role of memory B cells and plasma cells. Levels of $CD8^+CD44^{hi}CD62L^{low}$ memory cells were significantly higher in immune mice as compared to malaria naïve controls which were not further boosted on challenge. Immune profiling of spleen cell phenotypes indicated that $\alpha\beta$ T cells, $CD4^+$ T cells, $\gamma\delta$ T cells and $Ly6G^+$ neutrophils were significantly higher in post challenged mice as compared to infection cleared mice and age matched controls suggesting a possible role of these immune cell subsets in mediating resistance. Furthermore, frequency of splenic ($CD69^+$) $CD4^+$ and $CD8^+$ activated T cells was significantly higher in post-challenged mice as compared to infection cleared mice and age matched controls indicating that reinfection of BALB/c mice with malaria parasites induced significant T cell activation in spleen. Lastly, the percentage of ($IL10^+$) $CD4^+$ T cells was significantly higher in infection cleared and post challenged mice indicating the existence of anti-inflammatory immune response in these mice. The details of these results and relevance in clinical immunity in endemic areas will be discussed.

664

PROTEIN MICROARRAYS REVEAL DIFFERENTIAL ANTIBODY REACTIVITY TO THE *PLASMODIUM FALCIPARUM* PROTEOME IN CHILDREN WITH SYMPTOMATIC MALARIA IN WESTERN KENYA

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Naturally acquired immunity (NAI) to *Plasmodium falciparum* (Pf) is characterized by age-related control of *parasitemia* and protection from

clinical malaria. With the goal of advancing knowledge of how the magnitude and breadth of anti-Pf IgG antibodies (Ab) contribute to NAI, we used plasma from 100 adults (≥18 years) and 100 children (1-14 years) who participated in a treatment time to infection study in western Kenya to probe Pf protein microarrays that represent ~23% of the Pf proteome. (Antigen Discovery, Inc., Irvine, CA) Heat maps of arrays probed with adult and child Ab obtained before anti-malarial cure of *parasitemia* showed that there was significant overlap in the hierarchy of proteins recognized by Ab from both age groups. Adult Ab responses were stronger and reacted with a greater breadth of proteins than those of children. Kaplan-Meier survival analysis for time to infection over 11 weeks of observation showed no correlation with the strength or breadth of Ab responses. In contrast, the strength and breadth of Ab responses were weaker and narrower among children with symptomatic malaria compared with asymptomatic children and adults. Principal component analysis showed clustering of Ab responses to malaria protein subsets among symptomatic children relative to asymptomatic children and adults. Those antigens with significantly greater reactivity in protected than unprotected children (p-values ≤0.01 with Benjamini-Hochberg correction for false positives) included MSP10, MSP1, MSP2, LSA3, several PfEMP1's, ring exported protein-1 and sporozoite threonine and asparagine rich-protein. We conclude that Pf protein microarrays offer insight into targets of NAI and identify potential candidates for inclusion in multi-antigen malaria vaccines.

665

CHANGES IN THE LEVELS AND AVIDITIES OF ANTI-MALARIAL ANTIBODIES IN MALIAN CHILDREN OVER THREE TRANSMISSION SEASONS

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There is a general consensus that high levels and broad specificities of antibodies to blood-stage proteins of malaria parasites are essential in reducing the susceptibility to clinical malaria. However, sero-epidemiologic studies to characterize the quantitative and qualitative changes in antibody responses and establish associations with clinical malaria incidence have yielded conflicting results. To address these issues, we took advantage of a well-characterized 3-year longitudinal sero-epidemiological cohort to 1) evaluate the dynamics of seasonal changes in antibody levels and avidities, and 2) assess whether such changes are associated with reduced risk of clinical malaria. The cohort included 3- to 11-year old Malian children (n=240) living permanently in a region where malaria is endemic and seasonal. Plasma samples were collected from the same child before and after each transmission season and all clinical malaria episodes were documented. The levels of IgG specific for four merozoite antigens (AMA1, MSP1, MSP2 and EBA-175) were measured by a standardized ELISA and antibody avidities measured by Surface Plasmon Resonance. While antigen-specific IgG levels generally increased during wet seasons and then decreased during subsequent dry seasons, the magnitude of change in IgG levels among individuals fluctuated from year to year depending on the antigen tested. Children with high IgG titers exhibited greater changes in IgG levels than those with low IgG titers. We observed some seasonal fluctuations in avidities of antigen-specific antibodies among children but observed no cumulative increases in these avidities over the course of the entire three consecutive transmission seasons. There was no association between the magnitude of changes in IgG levels or avidities and reduced risk of malaria. Nonetheless, our findings underscore the need for conducting multi-season longitudinal studies and evaluating multiple antigens in order to identify malaria-protective antibody responses.

666

IL-15 MEDIATED SURVIVAL OF INTRAHEPATIC CD8 CENTRAL MEMORY CELLS IS ASSOCIATED WITH LONG-LASTING PROTECTION AGAINST MALARIA INFECTION

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Ag-specific memory T cell responses elicited by infections or vaccinations are inextricably linked to long-lasting protective immunity. Studies of protective immunity amongst residents of malaria endemic areas indicate that memory responses to *Plasmodia* antigens are not adequately developed or maintained. In contrast, multiple exposures to radiation-attenuated Plasmodia sporozoites (γ-spz) induce long-lasting protective immunity to experimental sporozoite challenge. We have previously reported that multiple exposures to *P. berghei* γ-spz (Pb γ-spz) confers protection in B6 mice. Protection in this model is associated with the accumulation of intrahepatic CD8 T cells comprising two major subsets ($T_{E/EM}$ and T_{CM}); while CD8 $T_{E/EM}$ cells are the primary producers of IFN-γ, CD8 T_{CM} cell display high expression of CD122 (IL-15Rβ). IL-15 is well known for its role in influencing the composition of the memory CD8 compartment through regulation of homeostatic proliferation, survival and differentiation into effector populations. To study the essentiality of IL-15 and the role of CD8 T_{CM} cells in protection, we immunized IL-15 KO mice with Pb γ-spz and discovered that, in spite of having reduced numbers of CD8 T cells, these mice are able to respond normally to *Plasmodium* antigens by expanding CD8 $T_{E/EM}$ cells and are protected short-term against a primary challenge with wild-type sporozoites. However, protection is short-lived, owing to reduced expression of Bcl-2 and increased apoptosis of proliferating intrahepatic CD8 T_{CM} in the absence of IL-15. Therefore, we hypothesize that the maintenance of long-lasting protection induced by Pb γ-spz depends on a process whereby intrahepatic CD8 T_{CM} cells, maintained by IL-15-mediated survival and basal proliferation, are conscripted into CD8 $T_{E/EM}$ cell pool during subsequent infections.

667

IDENTIFYING KEY TARGETS OF ANTIBODIES TO *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES USING GENETICALLY-MODIFIED PARASITES WITH DISRUPTED SURFACE ANTIGEN EXPRESSION

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Effective clinical immunity that protects against symptomatic malaria in humans develops gradually after repeated exposure to *Plasmodium falciparum*. During intra-erythrocytic development, *Plasmodium falciparum* expresses novel antigens on the surface of infected erythrocytes, including PfEMP1, RIFIN, STEVOR, and SURFIN. Antibodies to surface antigens are typically variant-specific and appear to play an important role in contributing to protective immunity in humans. However, the significance of different surface antigens as targets of acquired immunity remains unclear. In our study, we used an innovative approach to evaluate the importance of surface antigens as antibody targets. This was achieved using genetically-modified *P. falciparum* lines with disrupted parasite protein trafficking, achieved by deletion of the skeletal-binding protein 1 (SBP1-knockout), and *P. falciparum* lines with inhibited expression of PfEMP1 (PfEMP1-knockdown). Currently, only PfEMP1 is known to be trafficked via the SBP1-pathway to the infected erythrocyte surface. We used high-throughput flow cytometry-based assays to measure antibody reactivity to the infected erythrocyte surface and opsonic phagocytosis

assays using samples from cohort studies in Papua New Guinea and Kenya, including children, adults, and pregnant women. Comparison between the parental and genetically-modified parasites allowed us to quantify the proportion of antibodies targeting specific antigens on the infected erythrocyte surface. We found very little antibody response to SBP1-knockout parasites and markedly reduced antibody response to PfEMP1-knockdown parasites. Our results from studies using multiple parasite lines in two geographically different populations suggest that the major surface antigens targeted by human antibodies are dependent on SBP1 for trafficking to the infected erythrocyte surface and are consistent with PfEMP1 being the dominant target of acquired antibodies. These findings enhance our understanding of acquired immunity to human malaria and have significant implications for vaccine development.

668

CELLULAR IMMUNE RESPONSES TO A NOVEL MALARIA VACCINE CANDIDATE, PF SCHIZONT EGRESS ANTIGEN-1, IN YOUNG CHILDREN AND ADULTS

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We discovered Pf Schizont Egress Antigen-1 (PfSEA-1) using a differential screening approach with plasma from children who were resistant or susceptible to *falciparum* malaria. Antibodies to the immunorelevant region of PfSEA-1 (rPfSEA-1A, aa 810-1083) predict resistance to severe disease in two yr old children, block schizont egress from infected RBC *in vitro,* and vaccination with rPbSEA-1A protects mice from *P. berghei* ANKA challenge. To advance PfSEA-1 as a vaccine candidate, we have evaluated cellular immune responses to rPfSEA-1A using cryopreserved PBMCs collected from 3 yr old children and adults living in a holoendemic region of western Kenya. In *in vitro* stimulation assays, endotoxin free rPfSEA-1A induced up-regulation of pro-inflammatory and TH1 cytokines in both Kenyan children (n=19) and adults (n=5). rPfSEA-1A stimulated PBMCs from adults produced 1.76-30 fold higher levels of IFN-γ, IL-2, IL-6, IL-8, IL-12, and TNF-α compared to stimulated PBMCs from children (all $P<0.03$). We analyzed the T-cell effector/memory subsets producing these cytokines using multi-parameter flow cytometry. The frequency of $CD4^+$ T cells making IFN-γ in response to rPFSEA-1A stimulation was 2.77 fold higher in adults compared to children and was largely produced by T-central memory ($CD45RA^{hi}$, $CCR7^{hi}$) and T-effector/memory ($CD45^{low}$, $CCR7^{hi}$) in adults but by T- naïve cells in children ($CD45RA^{hi}$, $CCR7^{hi}$). These data confirm that rPfSEA-1A contains broadly reactive T cell epitopes, rPfSEA-1A specific T-cell responses are detectable in young children, and the frequency of these responses increases with age due to natural exposure. We plan to relate rPfSEA-1A cellular responses and resistance to *falciparum* infection in a larger longitudinal study of Kenyan children.

669

PLASMODIUM VIVAX DUFFY-BINDING PROTEIN-SPECIFIC MEMORY B CELL FREQUENCIES CORRELATE WITH FUNCTIONAL ANTIBODIES TO PVDBPII AND PERSIST IN AREAS OF LOW MALARIA TRANSMISSION

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Plasmodium vivax (Pv) Duffy-binding protein (PvDBP) engages the reticulocyte surface and is critical for red cell invasion. Naturally-acquired, binding-inhibitory antibodies (BIAbs) to PvDBP correlated with reduced risk of Pv infection in Papua New Guinea. Serum samples from a western Cambodian study of acute Pv malaria episodes revealed high IgG responses to PvDBP antigens that were both strain-transcending and strain-specific. Specificity can change with one amino acid substitution in PvDBP. The basis of persistent PvDBP-specific memory B cells (MBCs) and their relationship to BIAbs, PvDBP-variant specificity, and total antibodies to PvDBP ($PvDBPAb_{tot}$) have not been examined. Sera were screened for BIAbs and $PvDBPAb_{tot}$ *in vitro* by bioplex/inhibition ELISA [N=189, median (range) age = 23 (2-68) years]. All samples had detectable levels of $PvDBPAb_{tot}$ and 4.7% of them had 'high' BIAbs (>80% binding inhibition relative to malaria-naïve donors). At various times [median (range) = 33 (6-45) months] after their acute Pv malaria episode, sera and PBMCs were collected from 20 adults [median (range) age = 28 (20-54) years] with high (N=9), moderate (N=1), and low (N=10) BIAbs. Four of 9 (44%) individuals retained high BIAbs and one individual without previously-detectable BIAbs acquired high BIAbs. $PvDBPAb_{tot}$ levels dropped by 2 to14-fold in 65% of individuals. By ELISPOT, the median (range) frequency of PvDBP-specific MBCs/10^6 PBMCs was 112 (17-976) and comparable to frequencies of MBCs specific for $PvMSP1_{19}$ (median=126) and tetanus toxoid (media=176). The frequency of PvDBP-specific MBCs correlated with BIAb ($r^2=0.48$, $p=0.0007$) but not with $PvDBPAb_{tot}$ levels. The data suggest that a Pv malaria episode induces PvDBP-specific MBC levels that correlate with BIAb levels and persist for months in areas of low Pv endemicity. Thus, PvDBP-specific MBC frequencies may represent a biomarker of immunologic memory to Pv malaria. This study also indicates that functional Abs can wane with low Pv exposure, which may account for the slow acquisition of immunity in some individuals.

670

SEROPREVALENCE TO MALARIA PARASITES IN TAK PROVINCE, THAILAND REVEALS MORE FREQUENT EXPOSURE TO *PLASMODIUM SP.* THAN ESTIMATED BY EPIDEMIOLOGICAL SURVEYS

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Malaria is the most important vector-borne disease in Southeast Asia. In Thailand, malaria incidence has been in decline, with the annual parasite incidence dropping to 0.56 in 2007. The Myanmar-Thai border province of Tak is considered mesoendemic for malaria, and both *Plasmodium vivax* (Pv) and *P. falciparum* (Pf) are equally present. As part of the International Centers for Excellence in Malaria Research (ICEMR) - Southeast Asia project, malaria surveillance is conducted in Tak on both the healthy population and hospital patients, and parasite prevalence is reportedly between 1-2%. However, still little is known about the immuno-epidemiology associated with Pf and Pv infections in the region regarding the breadth and targets of the antibody response to the malaria parasites. Our hypothesis is that the serological profiles of the population will reflect the low parasite prevalence in Tak, showing little antibody reactivity to Pv and Pf. To examine this question, we developed a protein microarray displaying the top 500 most immunogenic antigens of these two *Plasmodium* species. Because malaria prevalence is low, we collected whole blood samples from febrile suspected malaria patients to increase the chances of detecting antibody responses. The sera was probed on the microarray and compared to healthy blood donors from the U.S.; genomic DNA was extracted from RBC pellets and screened by PCR for infection confirmation and species-identification. We detected 14 Pv+ (23%), 6 Pf+ (10%), 2 mixed infections (4%) and 38 (63%) PCR-negative samples. Seventy percent (42 of 60) of serum samples were highly reactive to both Pv and Pf antigens, surprisingly including 22 of 38 (57%) PCR-negative samples. Serum from individuals with Pf+ PCR always produced seropositive profiles on the array, whereas serum from Pv+ individuals produced both seronegative (35%) and seropositive (65%) profiles.

Despite the low detectable Pv and Pf infection prevalence in Tak province, there is unexpected substantial antibody reactivity to the malaria parasites, even amongst non-infected individuals.

671

POLYMORPHISMS IN CO-STIMULATORY GENES DO NOT AFFECT *PLASMODIUM VIVAX* PARASITE DENSITY

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Plasmodium vivax is the most prevalent malaria species in Brazil, representing more than 80% of clinical cases reported annually from the Amazon region. A growing body of evidence indicates that the immunity is important in the outcome of *P. vivax* infection. Co-stimulation is an important secondary signal that governs the extent, strength and direction of the immune response that follows. Since parasite density has been recognized as important factor in the outcome in malaria infections, we investigated whether polymorphisms in co-stimulatory genes are associated with *P. vivax parasitemia* in malaria patients from Brazilian Amazon Region. The sample included 147 patients infected with *P. vivax* from Goianésia do Pará, a municipality situated on the southwest of Pará state, Brazil. Nine SNPs were analyzed by PCR-RFLP in seven co-stimulatory genes (BAFF, CD28, CTLA4, CD40, CD40L, CD86 and ICOS). Parasitemia was determined by counting the number of parasites in 100 separate fields under oil immersion microscope and converted to the number of parasites per microliter of blood assuming 8,000 leukocytes/µL. Association between the genotypes and parasite density was determined by Mann-Whitney test, with level of significance of 0.05, using R statistical software. All SNPs tested were in Hardy-Weinberg equilibrium. A trend was noted between the allele C of SNP rs_3116496 in CD28 gene and lower parasite density, but these trend was not significant ($p = 0.1$). No significant association was found between the polymorphisms tested and *P. vivax* parasite density. Our results show that the studied polymorphisms do not affect the *P. vivax* parasite density. However, due to the obvious importance of co-stimulatory pathways in malaria, further studies that elucidate the complex host-parasite interactions could be useful for future vaccine development.

672

MERCURY LEVELS IN HAIR OF WOMEN IN A NATIVE COMMUNITY, MADRE DE DIOS, PERU: AN ORIGINAL RESEARCH

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Mercury originates neuropsychological disturbances in neonates exposed intrauterine. In addition, as a result of the emergence of illegal gold mining, there has been an increasing level of pollution in the River Madre de Dios, Peru. Not knowing statistically significant data on pollution in women of childbearing age of native communities, we are determining the levels of mercury present in the food chains in women of childbearing age in the native community Ese'eja Palma Real. An observational, descriptive, cross-sectional study. Samples were collected from the hair of women in fertile age (11- 44 years), which were analyzed by atomic absorption test cool mist in the center of toxicological SAC. Sociodemographic information was collected by a questionary. The analysis was performed using the statistical package STATA 11.0 (STATA Corp®, Texas, US),likewise frequencies were used, measures of central tendency and dispersion for qualitative variables. An important percentage of the population 33.33% (n = 20) of the women showed figures of mercury in hair higher than 2 mg/g. Levels were found above the permissible limit of mercury in hair in women childbearing of the native community Ese'eja Palma Real, Madre de Dios, Peru.

673

COMPARISON OF TWO COMMUNITIES AFFECTED BY CHOLERA IN KASESE DISTRICT IN UGANDA

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Some sub-counties in Kasese District, experienced frequent cholera outbreaks since the year 2000 to-date, while others did not. The reasons for this difference were not entirely clear nor had they been explored. This study was therefore carried out to try to establish factors why cholera outbreaks were frequent in some areas and not others of the same district. The specific objectives were to study the socio-demographic profiles of the residents of two sub-counties, assess their socio-cultural practices, their environmental sanitation situations as well as the water sources used, cholera carrier status and the antibiotic sensitivity of the cholera organisms responsible for the cholera outbreaks. A cross-sectional study comparing the situations of residents of Karambi and Bugoye was carried out. Focus group discussions, Interviews and observations of the homesteads, latrines and markets for sanitation and hygiene were conducted; Water samples from sources and households were analysed for faecal contamination. Similarly stool samples obtained from victims who had recovered from cholera were cultured and isolated cholera organisms, tested for sensitivity to first line antibiotics. The main findings why cholera remains problematic in Karambi included: poor hygienic practices, (difference significant $p \leq 0.004$),as well as eating communally from the same dish (difference significant $p \leq 0.008$) and unsafe water sources, contaminated with faeces (both *E. coli* and cholera organisms were isolated from R. Mbabaine of Karambi). In Karambi, 36% of the cholera victims who had recovered were found to be asymptomatic carriers of cholera organisms, resistant to first line antibiotics. Factors therefore responsible for the difference in cholera outbreaks in the two communities were: Unsafe water sources, poor hygienic practices; and high carrier status among the Karambi community with cholera organisms resistant to first line antibiotics.

674

CHOLERA FROM THE LENS OF HOUSEHOLD HEADS: PERCEIVED SUSCEPTIBILITY AND HYGIENE PRACTICES IN CHOLERA-INFECTED AND NON-INFECTED COMMUNITIES IN IBADAN NORTH WEST LOCAL GOVERNMENT AREA, NIGERIA

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Cholera is a re-emerging disease of public health challenge where water supplies and sanitary conditions still constitute problems. This study therefore designed to determine household heads perceived susceptibility to cholera and hygiene practices in Cholera Infected Communities (CIC) and Non-Infected Communities (NIC) in Ibadan North West Local Government Area, Nigeria. This is a descriptive comparative study. A four-stage sampling technique involving purposive sampling was used in selecting heads of households used for this study. A total of 800 respondents (400 each) were used for this study in both CIC and NIC in IBNW. Interviewer-administered questionnaire and Focus Group Discussion (FGD) were used for data collection. A 7-point scale was used to measured respondents' perceived susceptibility to cholera. Descriptive statistics were used for quantitative data while FGD were subjected to thematic analysis. Mean age of household heads were 48.5±11.8 and 47.0±11.5 years in CIC and NIC respectively. Some (26.7%) and 14.0% of the respondents in CIC and NIC respectively were of the believed that they are at risk of getting cholera. FGD participants also perceived themselves high to cholera due to poor state of their environment. Majority (93.1%) and

91.9% in CIC and NIC respectively were of the perception that cholera can be gotten from spoilt food. Many (56.2%) and 46.8% were of the opinion that ORS can cure any infected person in CIC and NIC respectively. Only (49.5%) and 46.5% of respondents in CIC and NIC respectively usually treat their water before drinking. Few (4.7%) and 11.3% of respondents in CIC and NIC respectively usually use water guard in treating their water. Only (0.9%) and 0.0% of respondents in CIC and NIC respectively are using dispenser for storing water. Respondents' perceived themselves susceptible to cholera infection yet inadequate preventive practices exist. Community health education on proper hygiene should be intensified in the respondents' communities.

675

RELATIONSHIP OF FOOD HANDLERS' KNOWLEDGE AND BEHAVIOR TO *ESCHERICHIA COLI* CONTAMINATION ON FOOD SERVING IN CAFETERIA A CAMPUS

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The previous studies showed that foods serving in cafeterias around the campus contaminated by *Escherichia coli.* This research was to know the relationship between the knowledge and behavior of food handlers serving foods in campus and the contamination of *E. coli.* The cross sectional design used to interview 173 food handlers of all cafeterias and foods serving were as samples. Most Probable Number (MPN) used in analyzing *E. coli* in foods conducted in the Laboratory of Environmental Health, Faculty of Public Health, Universitas Indonesia. Chi square test and logistic regression tests used to analyze the data collected. The result showed that a total of 59.54 foods contaminated by *E. coli.* Poor knowledge of food handler in serving food was statistically significant relate with *E. coli.* contamination (p= 0.008; OR= 2.42; CI 95%: 1.30-4.51) and behavior in washing hands had relation with *E. coli.* contamination as well. (p= 0.022; OR= 0.106; CI 95%: 0.01-0.83). Logistic regression found that poor knowledge in serving foods and behavior washing hands before preparing foods were factors contribute in *E. coli.* contamination in foods with OR = 2.70 (CI 95%: 1.42-5.11; p= 0.002) and OR= 0.08; CI 95%: 0.01-0.65; p=0.018) respectively. Poor knowledge of food handler in serving foods was a risk factor, whereas washing hands before serving foods was a protective factor. Food handlers should educate and give training to raise their knowledge and have a proper knowledge and practice in food serving and preparation.

676

SHARED SANITATION AND DIARRHEAL DISEASE: EVIDENCE FROM THE DEMOGRAPHIC AND HEALTH SURVEYS AND RURAL ECUADOR

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The WHO/UNICEF Joint Monitoring Program (JMP) defines all shared toilet facilities as unimproved, regardless of the level of technology. The JMP's sanitation ladder is a 4-tier categorization scheme and differentiates between improved facilities and facilities that are shared but otherwise improved. However, there is little evidence to corroborate this policy and even less understanding of the underlying mechanisms that could lead to elevated risk of diarrhea. Using data from 51 Demographic and Health Surveys completed since 2001, we assess whether the prevalence of childhood diarrhea is higher among those with shared facilities. We also compare the prevalence of diarrhea across levels of the JMP sanitation ladder, as well as by the magnitude of sharing (the number of households that share the facility). In the majority of countries, the prevalence of diarrhea was higher among those with shared facilities than among those with a facility that is not shared. The crude prevalence ratio pooled across all 51 countries was 1.10 (95%CL: 1.08-1.14), but it was substantially attenuated after adjusting for confounders (PR=1.02, 95%CL: 1.00-1.05). Sharing appears to be a risk factor in many countries, but confounding plays an important role. The effect of sharing varies widely across countries, making global policy related to this issue challenging. We are currently conducting fieldwork in rural Ecuador to better characterize sharing and its effect on the transmission of diarrheal pathogens.

677

ARE THERE CHANGES IN DRINKING WATER MANAGEMENT FOR YOUNG CHILDREN DURING A CHOLERA THREAT IN A POOR PERI-URBAN COMMUNITY IN THE DOMINICAN REPUBLIC?

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Water-linked disease outbreaks typically prompt increased promotion of the importance of safe drinking water with anticipation of improved purification practices where needed. However, there are few reports of changes in household level water management practices in response to such outbreaks. This study aimed to determine changes in drinking water purification for young children by caregivers over time in relationship to a threat of cholera. Household level drinking water management strategies for young children (0-5 years of age) were extracted from caregiver reports obtained through repeated interviews over time at appointments within a pre-existing and ongoing child growth monitoring program in a poor peri-urban community in the Dominican Republic. Patterns of drinking water improvement practices (chlorination, boiling, use of bottled water) over time relative to the timeline of a cholera outbreak were determined. Caregivers (mostly mothers) of 204 children provided 806 data points on drinking water practices between Sept 30, 2010 and July 10, 2012. The first cholera case in neighbouring Haiti was reported in Oct 2010, then in the Dominican Republic in Nov 2010, and then in the study community in April 2011. Over the study period, use of bottled water was the most frequent routine practice employed by child caregivers (52.6%), with infrequent reliance on boiling (12.8%) and household level chlorination (5.8%). No consistent changes in the employment of drinking water improvement strategies were identified over the period of time studied. However, there were short-lived increases in chlorine use in Feb-March, 2011 (to 15.6%) and bottled water use in April-May 2011 (to 78.1%) which also represented the peak use for these strategies during the study period. Further investigation is needed to determine child caregivers' perceptions of disease threat, beliefs about the value of drinking water improvement strategies, and the effectiveness or lack thereof of different health education strategies.

678

HOUSEHOLD-LEVEL INITIATED DRINKING WATER IMPROVEMENT STRATEGIES AND CHILD DIARRHEA IN A POOR PERI-URBAN COMMUNITY IN THE DOMINICAN REPUBLIC

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While evidence-based household-level strategies for improving drinking water quality are well known, comparatively little is known about the patterns of typically employed strategies in various settings. This knowledge gap is particularly concerning for high-risk populations in high-risk settings such as for young children in poor districts of low- and middle-income countries. Determining typically employed strategies and their relationship with child diarrhea may provide locally relevant data to inform health education. This study aimed to determine the drinking water improvement strategies employed for children (0-5 years of age) in a peri-urban community near Santo Domingo, Dominican Republic (DR) and examine whether strategies were related to child diarrhea. Caregivers attending a growth monitoring program, which enrolls most of the community's children, participated in standardized health interviews at each appointment which collected information on child diarrhea in the preceding 2 weeks and the frequency the child drank different types

of improved drinking water in the preceding 4 weeks. Caregivers of 199 children agreed to release their interview data. Multiple responses per child were adjusted by weighting. Approximately 22% of children had had diarrhea prior to their appointment (compared with a national prevalence value of 14% found in the most recent DR Demographic and Health Survey). "Always" and "sometimes" using bottled water (53 and 22%, respectively) were the most frequently reported practices, followed by boiling (12 and 11%) and chlorination (6 and 22%). The high levels of bottled water use are consistent with other reports on the DR. No reported strategy use was related to child diarrhea. Possible factors, requiring further investigation, which may explain this lack of relationship, include (i) reported practice not reflecting actual practice, (ii) inadvertent contamination of improved drinking water, and/or (iii) some situations whereby water improvement practices were employed in response to child diarrhea.

679

MICROBIAL CONTAMINATION ON PRODUCE AND FARM WORKER HANDS THROUGHOUT THE PRODUCTION PROCESS ON FARMS AND PACKING SHEDS IN NORTHERN MEXICO

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Although produce associated outbreaks are a serious public health burden, few studies have characterized contamination routes or points in the production process where contamination has occurred. Thus, we aim to: 1) quantify contamination by fecal indicators on produce and farm workers' hands at different points in the chain of events surrounding the production and post-harvest handling process, and 2) assess the association of microbial levels between matched produce and hand rinse samples. Produce rinses (160 cantaloupe, jalapeño, and tomato samples) and matched farm worker hand rinses (107) were collected from 9 farms in northern Mexico at four points: before and after harvest, at the point of distribution from the field, and at the packing shed. Generic *E. coli*, Enterococci, fecal *coli*forms, and *coli*phage were enumerated. Logistic regression found that the risk of E.*coli* presence on produce was 7 times greater when the matched handrinse had *E. coli*, (OR=7.1, 95% CI=2.3-21.6), and a significant increased risk was also seen with *coli*phage (OR=95.6, 95% CI=8.5-1076.4). Spearman's correlations indicated that the concentrations of *E. coli* (rho=0.9), Enterococci (rho=0.5), and *coli*phage (rho=0.7) were significantly correlated between hands and produce. Chi-square tests revealed that the prevalence of contamination on produce varied significantly across points in the chain for *E. coli* (χ2=21.9, p<0.001), Enterococci (χ2=23.5, p<0.001), and *coli*phage (χ2 =14.5, p=0.002). The prevalence of *E. coli* on produce increased from 28% before harvest to 67% at the packing shed, Enterococci increased from 69% to 90%, and *coli*phage from 38% to 61%. Hand hygiene and the packing shed environment should be targeted for effective interventions to mitigate the risk of microbial hazards on produce.

680

THE EFFECT OF CLIMATIC FACTORS ON CHOLERA INCIDENCE IN THE FAR NORTH REGION OF CAMEROON

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Cholera is considered as a model for climate-related infectious diseases. During the past 15 years, 11 outbreaks of cholera with varying intensity and spatiotemporal extend were reported in the Far North Region of Cameroon. These outbreaks occurred mainly during the rainy season, but it is not known how climate variability influences the incidence of cholera in this region. In this study, the variability pattern of cholera events was studied in association to local climate variables in one of the 30 health districts found in the Far North. We used monthly time series of total reported cholera cases, average monthly rainfall, average monthly temperature and average monthly relative humidity from 1996 to 2011 to explore the association between climatic factors and cholera incidence in the Maroua urban health District. A Generalized Additive Modelling (GAM) framework was used to assess the effect of climatic factors on cholera incidence. A stepwise single predictor approach and a multiple predictors approach were used. In the single predictor approach, it was found that the deviance explained by humidity for example was 19.7%; while the deviance explained by temperature was 26.8%. The results of the multiple predictors model without considering interactions give a deviance explained of 75.6% and 95.4% when considering the interaction between the factors. These results demonstrate that the occurrence of cholera is geared by the combination and interaction of rainfall, humidity and temperature which provide appropriate conditions for the development and spread of vibrion choleric.

681

TOTAL MERCURY CONCENTRATIONS IN SAMPLES OF MUSCLE OF THE FRESHWATER FISH, *ASTYANAX BIMACULATUS,* AND IN THE SEDIMENTS FOLLOWING THE FINAL COURSE OF CAPIM STREAM, MINAS GERAIS, BRAZIL

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Aquatic ecosystems around the world are increasingly impacted by heavy metal pollutants mercury. This element cannot be destroyed and, once in the food web, it reaches increasingly progressive concentrations known as bioaccumulation. The present investigation aims at assessing the degrees of total mercury accumulation, in samples of muscle of *Astyanax bimaculatus* (n=192) and in the sediments following the final course of Capim Stream, Minas Gerais, Brazil. *In situ* measurements in the fresh water collections allowed us to acquire data on the physical characterization of the rivers, from which we could infer the behavior of mercury in the stretch of the river under study. The determination of mercury was tested by using an atomic absorption spectrometer coupled with a cold steam-generating device. The stretch of Capim Stream was shallow with warm, buffered water, despite of the presence of slight acid/basic (pH=6.3/7.8) values in some sampled sites. The water has also shown to be little oxygenated (2-6mg/L), with increased values of total suspended solids (72-200 mg/L) and electric conductivity (90-743 µS/cm). Such conditions have enabled theadsorption of mercury to the total suspended solids and organic carbon as well the growth of algae in sites of steady flow of stream. Algae and sediments represent the food basis for *A. bimaculatus*.In the fish muscles, the highest values of mercury were quantified in the specimens collected in the water pond (1350ng/g^{-1}p.f.;1540 ng/g^{-1}p.f) in the late dry season, and in the mouth of the river (1070 ng/g^{-1}p.f.) in the late rainy season. The feeding seasonality of the Lambari and sedimentin both seasons, besides algae in summer has allowed us to suggest that these fish are contaminated with mercury during the feeding period. In general terms,the degrees of total mercury in the muscle of the specimens collected showed to be low (150-1070ng/g^{-1}p.f.), which are in accordance with compatible values for human consumption permitted by Brazilian Legislation (500 ng/g).

682

PERCEPTION OF CHOLERA OUTBREAK, ATTITUDE TO REPORTING AND INVESTIGATION AMONG COMMUNITY RESIDENTS IN IBADAN NORTH-WEST LOCAL GOVERNMENT AREA, NIGERIA

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Cholera has potential for outbreak and is a major threat to lives if not responded to early. Rapid containment of an outbreak is largely dependent on the attitude and behavior of the community as well as perceived risk. This study was conducted to determine knowledge of cholera and its control practices, perceived vulnerability and severity to cholera and attitude to reporting and investigation among residents of IBNW LGA, Nigeria. Cross-sectional design was employed. Three-stage random sampling technique was used to select 427 consenting household members aged ≥18 years old. Communities in the LGA were stratified into three groups (28 inner core, 15 transitory and 17 peripheral) and a quarter of the communities in each group was randomly selected. Households were visited and eligible members interviewed using a semi-structured questionnaire. Knowledge was scored on a 19-point scale (score of ≤10 rated poor and ≥11good), perceived vulnerability on 15-point (scores of ≤7 rated low and ≥8 high) while perceived severity was scored on 25-point (≤ 12 rated low and ≥ 13 high). A 24-point scale was used to score attitude to reporting of cholera outbreak (score of ≤ 12 rated negative and ≥ 13 positive). Data were analysed using descriptive statistics, Chi-square test, and logistic regression at p≤ =0.05. Respondents mean age was 35±11.4 years and 70.7% were females. Most (95.3%) of the respondents had good knowledge of cholera. About 71.4% knew the cause of cholera while 97.2% and 96.3% knew diarrhea and vomiting as clinical sign respectively. The commonest source of information during an outbreak was the radio (38.6%). Many respondents (62.3%) perceived their vulnerability to cholera to be low while majority (98.1%) perceived severity of cholera infection to be high. Significantly, respondents residing in the inner core communities perceived themselves more vulnerable to cholera outbreak (OR=23.7: 95%CI 9.64-58.31). Most (71.2%) of the respondents had positive attitude to reporting of cholera outbreak. The good knowledge of cholera, perception of its severity as high and positive attitude to reporting of cholera among this study participants offers a good ground to address more specific risks issues aiming at improving hygiene practices and low perceived vulnerability.

683

SUSTAINABLE ACCESS TO SAFE WATER IN HONDURAN HOSPITALS

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Centralized distribution systems improve access to water quantity; however, ensuring water quality is difficult in low-resource settings. Point-of-use water treatment is widely promoted to improve water quality, but is difficult to sustain. Decentralized treatment can provide safe water without reliance on point-of-use products and individual water treatment behavior. Membrane filtration systems are being adopted in a growing number of low-income settings. There is a need to understand the long-term sustainability of these systems. Between 2007-09, General Electric Foundation donated membrane-filtration systems to 4 hospitals in Honduras. In 2012-13, the Center for Global Safe Water at Emory University visited these sites to assess the technological, organizational and contextual factors that affect sustainability. At each hospital, 30 water samples were analyzed for *E. coli* and 25 safe water knowledge, attitude and practice surveys were conducted with staff and patients. These same data were collected at two control hospitals to assess the impact of the treatment systems. Water from hospitals with treatment systems had significantly lower *E. coli* concentrations than hospitals without these systems. Staff in hospitals with treatment systems were more likely to believe the water was safe to drink (24% vs 0%, p=0.02); but there was no difference in the percentages of staff who drank the water (24% vs 11%, p=0.60). We developed a metric to evaluate the sustainability of the systems using 4 domains: on-site capacity, accountability, technical feasibility, and institutional engagement. Domains were scored from 0 to 4 (4 being the most sustainable) based on interview responses and laboratory results. A score of 2 was defined as the cutoff for sustainability. Domain scores showed different strengths and weaknesses related to sustainability. Overall, the hospitals scored near the cutoff (from 1.7-2.4). The domain of technical feasibility had the lowest scores. The treatment systems were often bypassed due to pressure and flow issues within the piped network. Hospitals also lacked access to and funds for critical parts. On-site capacity scores were high but could be improved through increased training and communication. Efforts to support the sustainability of institution-based water treatment devices should focus on improving training and communication as well as assessing technical feasibility prior to implementation.

684

THE COMPARTMENT BAG TEST AS A WSH HEALTH BEHAVIOR INTERVENTION TOOL IN MWANZA, TANZANIA

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Microbial contamination is not detectable with the naked eye so the link between water and disease is often not strong enough to influence behavior. Thus, a direct test for contamination of household water may be more effective than solely social marketing messages. Since much of water quality is determined by household treatment and storage, it is important to understand how household knowledge influences action. The Compartment Bag Test (CBT), a liquid culture quantal assay for *E. coli*, was evaluated in this study as a WSH health behavior and educational tool. Forty households in the urban and peri-urban areas of Mwanza, Tanzania participated in a semi-quantitative survey regarding drinking water attitudes and practice. On day 1, households were asked questions regarding water source, treatment, and storage practices and analyzed 100ml of the household drinking water with the CBT under supervision. After incubation for 18-24 hours at 37C, test results were reported back on day 2 to the household and a post-survey was conducted regarding attitudes and reaction. Perception of the safety of drinking water seems to be a key indicator of whether water is treated prior to consumption, since about the same percentage of respondents that perceived their water to be unsafe to drink, also treated their water prior to consumption, 57%. A statistical difference was found in the perception of safety before and after use of CBT (p-value=0.02). A logistic regression was run to determine the magnitude and direction of change. The perception of safety decreased 8.33 times, even when household drinking water quality was found to be safe according to WHO drinking water guidelines; the perception of safety decreased 16.67 times, when household drinking water quality was found to be unsafe. All users say they would recommend the use of the CBT and after seeing results, and 86.7% said they would change treatment practice. The CBT is a potentially useful health behavior and education tool that should be explored further in future WSH interventions.

685

PREDICTORS OF FECAL EXPOSURE IN URBAN PUBLIC LATRINES: THE ROLE OF CHARACTERISTICS AND CONDITIONS

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The Millennium Development Goals seek for countries to halve, by 2015, the proportion of people who lack sustainable access to an improved sanitation facility. An "improved" facility is defined as one that ensures hygienic separation of users from human excreta. Characteristics such as flooring or type of design are used as indicators that a household facility can meet that criteria. Facilities that are shared by >2 households are universally considered "unimproved", out of concern that more users can results in poorer hygienic conditions. Shared public latrines are frequently the only feasible option for meeting the sanitation needs of millions living in crowded urban low income cities. There is little evidence that shared facilities pose a greater exposure risk to users than private facilities, nor to describe the characteristics that might influence that risk. We hypothesized public latrines with permeable flooring, a lack of adequate septage containment, and heavier use would be more likely to contain visible feces and microbial contamination. We collected data on characteristics (typology, flooring material, presence of hand washing stations, access, crowding, safety, privacy, and management), conditions (visible feces, flies, smell), and microbial contamination (*E. coli*, enteric viruses) from 29 public latrines in 4 neighborhoods with varying population density and wealth in urban Accra, Ghana. Concentrations of *E. coli* in soil in the vicinity of the facilities (n= 16, mean 104/gram) and on surfaces inside the facilities (n=69, mean 103/100 cm2) were 1-2 logs greater than what has been reported in household latrines of comparable technology. We found no difference (P<0.05) in concentrations of *E. coli* and enteric viruses for flush/pour-flush and bucket latrines, permeable and impermeable flooring, and other characteristics, nor for neighborhood. These results suggest that public latrines in urban environments do pose a risk of exposure to feces and infectious viruses, but exposure is highly variable and cannot be predicted using commonly-observed indicators.

686

ARGININOLYSIS AND URICOLYSIS IN *AEDES AEGYPTI* MOSQUITOES REGULATE THE METABOLISM OF UREA AND OTHER NITROGEN WASTE PRODUCTS VIA A CROSS-TALK SIGNALING MECHANISM

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We previously demonstrated that blood-fed *Aedes aegypti* are able to fix, assimilate and excrete nitrogen very efficiently by using multiple metabolic pathways. During this metabolic challenge, female mosquitoes excrete several nitrogen waste products. Two metabolic origins have been proposed for the urea production, either from argininolysis or from uricolysis. We have recently shown that the expression level of arginase (AR) in tissues increases when urate oxidase (UO) is silenced by RNAi, and vice-versa, suggesting a cross-talk between the pathways. Since the blood meal digestion is delayed in mosquitoes injected with dsRNA-AR, dsRNA-UO or both (dsRNA-ARUO), we decided to examine the effect of knockdown on ovarian development by monitoring the vitellogenin levels in the ovaries by western blotting. The data indicate that the uptake of the vitellogenin by the ovaries of the AR, UO or ARUO dsRNA-injected females occurs at a lower rate during the first 48 h after feeding. Mosquitoes injected with dsRNA against AR and UO completed digestion and matured their oocytes by 72 h after feeding. The transient delay in both digestion and vitellogenesis led us to hypothesize that the synthesis and/or excretion of nitrogen waste regulate the expression of genes involved in fixation, assimilation and excretion of ammonia in *A. aegypti*. To verify this hypothesis, the expression patterns of the genes encoding glutamine synthetase, glutamate synthase, glutamate dehydrogenase, alanine aminotransferase, pyrroline-5-carboxylate synthase, pyrroline-5-carboxylate reductase and xanthine dehydrogenase were investigated in fat body from dsRNA-injected females after blood feeding. The silencing of AR, UO or ARUO expression led to a large decrease in the mRNA levels for most of the genes studied. These data demonstrate that the metabolism of nitrogen waste in mosquitoes is finely regulated by a complex cross-talk mechanism. By using this molecular mechanism, blood-fed female mosquitoes control the disposal of excess nitrogen without affecting their survival.

687

PHYSIOLOGICAL, MORPHOLOGICAL AND HORMONAL VARIATION IN *ANOPHELES GAMBIAE S.L.* MOSQUITOES EXPOSED TO THE STRESSFUL CONDITIONS OF THE DRY SEASON IN BURKINA FASO, WEST AFRICA

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In tropical savannahs of West Africa, mosquitoes have to cope with extended periods of harsh environmental conditions during the long (6-9 months) dry season. However, their survival mechanisms under aridity and drought remain poorly understood. This study explored the degree of physiological, morphological and hormonal changes that are being prompted by a switch between the rainy and dry season conditions in three members of the *Anopheles gambiae s.l.* complex that coexist in Burkina Faso. Insects were reared in climatic chambers reflecting environmental conditions recorded in the field during the rainy and/or the dry season. Their metabolic fingerprinting and proteins expression were analyzed by gas chromatography - mass spectrometry and 2D-DIGE respectively. Ecdysteroid hormones were quantified using an enzyme immunoassay and finally spiracles were observed under scanning electron microscopy (SEM). Our study revealed that older female mosquitoes reared under dry season conditions were characterized by lower concentration of tricarboxylic acid cycle intermediates and isoleucine, suggesting metabolic and reproduction depression in the dry season conditions. Overexpression of proteins involved in muscles' contraction (myosin light chain) and cuticle thickness and rigidity (cuticular proteins) were observed during the dry season in both An. coluzzii and *An. gambiae*. On the other hand An. coluzzii and An. *arabiensis* considerably reduced their spiracles apertures which are surrounded with high number of trichomes in dry season. Ecdysteroid concentration was much higher in males than in females, suggesting a role of these hormones in shaping *An. gambiae* reproductive strategies and population demography. By exploring physiological and morphological correlates of mosquito local adaptation, our work contributes to unraveling the complex mechanisms underlying the enormous adaptive potential hidden within the *An. gambiae* species complex.

688

CURING DENGUE VIRUS INFECTION IN ADULT *AEDES AEGYPTI* BY CHEMICAL INHIBITION OF HOST FACTORS

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Dengue virus (DENV) has become an increasingly important arbovirus transmitted mostly by the *Aedes aegypti* mosquito. Despite the public health burden of dengue, no vaccines or registered drugs are currently available. Thus, there is growing interest in targeting mosquito vectors to control the virus. Studies have identified dengue virus host factors (DVHFs) that allow the virus to infect the mosquito as well as the vertebrate host. Inhibition of these DVHFs in mosquitoes may represent an alternative method to reduce DENV transmission. Here we investigated whether known mammalian DVHF inhibitors can also reduce virus titer in the arthropod vector, *Ae. aegypti*. We applied two known chemical compounds (bafilomycin and mycophenolic acid) to mosquitoes using various treatment methods including injection, topical treatment or sugar and blood co-feeding. By injection, we confirmed that bafilomycin and mycophenolic acid inhibited DENV in the midgut at 7 days post infection by 55% and 66% respectively. It is known that bafilomycin binds to the highly conserved vATPase c subunit of V0 and mycophenolic acid binds to inosine-5′-monophosphate dehydrogenase in human cell lines. We will continue to study anti-DENV effects of the compounds in *Ae. aegypti*.

689

STRONG SELECTIVE PRESSURE AGAINST A RECOMBINANT SINDBIS VIRUS THAT INDUCES APOPTOSIS IN THE MOSQUITO *AEDES AEGYPTI*

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Our laboratory recently reported that *Aedes aegypti* mosquitoes which were injected with dsRNA corresponding to the anti-apoptotic gene *Aeiap1* exhibited high levels of midgut apoptosis, and this resulted in enhanced replication and midgut dissemination of Sindbis virus (SINV) following an infectious blood meal. Similarly, silencing the initiator caspase *Aedronc* resulted in decreased SINV replication and dissemination. These results could be interpreted as indicating that apoptosis promotes SINV replication and spread. However, the gene silencing approach affects both infected and uninfected cells, and could have secondary effects. As an alternative approach, we have utilized an alphavirus transducing system to construct a recombinant SINV that induces apoptosis. Oral infection of *A. aegypti* with a SINV expressing the pro-apoptotic *Drosophila* gene *reaper* (MRE/Rpr) induced apoptosis in infected midgut cells, while control viruses with similar size non-coding inserts did not. Replication of MRE/Rpr was reduced and delayed compared to control virus at early time points, but the titers of the two viruses were similar by 7 days post infection (dpi). Sequencing of plaque-purified viruses obtained from mosquitoes infected with MRE/Rpr revealed that beginning at 3 dpi, the majority of MRE/Rpr viruses recovered had deletions that eliminated expression of *reaper*. However, all control viruses recovered from infected mosquitoes contained intact control inserts, even up to 7 dpi. Together these results suggest that there was strong selective pressure against viruses expressing Reaper, and indicate that if apoptosis is triggered in infected cells, it can reduce SINV replication in *A. aegypti*.

689A

A POTENTIAL ROLE FOR EFFECTOR CASPASES CASPS18 AND CASPS19 IN MIDGUT ESCAPE OF SINDBIS VIRUS IN *AEDES AEGYPTI*

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The midgut epithelium is the first target of arboviruses when they invade the arthropod vector. To establish a disseminated infection, arboviruses must cross the midgut basal lamina (BL), an extracellular layer that is secreted by epithelial cells and prevents passive diffusion by viruses. We are using Sindbis virus (SINV) and the mosquito vector *Aedes aegypti* to understand how arboviruses escape from the midgut and establish systemic infections. During baculovirus infection in lepidopteran larvae, midgut infection initiates a cascade of protease activation in which matrix metalloproteases (MMPs) activate effector caspases, leading to cleavage of BL proteins and remodeling of the BL lining tracheal cells associated with the midgut, which allows baculovirus to escape the midgut. We hypothesize that the MMP-caspase-BL remodeling pathway is also used by arboviruses to escape the mosquito midgut. Prime candidates for caspase involvement in midgut escape and BL remodeling are CASPS18 and CASPS19, effector caspase homologs related to *Drosophila* Decay. Although CASPS18 does not have enzymatic activity, it has been shown to act as a decoy caspase that is able to enhance the activity of CASPS19. The levels of CASPS18 and 19 transcripts and proteins in midgut were not altered by SINV infection, but CASPS19 was cleaved and activated in midgut following a blood meal. RNAi-mediated silencing of CASPS18/19 caused a decrease in midgut caspase activity, and also resulted in lower virus titers than control mosquitoes following an infectious blood meal containing SINV. Immunofluorescence using antisera specific for CASPS18 and 19 revealed that CASPS18 and 19 were expressed in tracheal cells associated with midgut. SINV was also found in tracheal cells in SINV-infected midguts, suggesting that SINV may use the tracheal system to establish systemic infection, and that CASPS18 and 19 may facilitate midgut escape of SINV.

690

IDENTIFYING BINDING PARTNERS OF AGDSCAM FOR ITS ANTI-*PLASMODIUM* ACTIVITY IN MOSQUITO

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The insect's immune system use limited numbers of germline-encoded pattern recognition receptors to recognize numerous pathogen-associated molecular patterns. Only after recognition, insects can activate defense responses to eliminate invading pathogens. The Down syndrome cell adhesion molecule (Dscam) was first discovered in *Drosophila* as a highly diverse axon guidance molecule. Subsequent studies showed it was also involved in invertebrate innate immunity. *Anopheles gambiae* Dscam (AgDscam) contains 10 Ig domains and 6 fibronectin repeat domains with the potential of generating over 31,000 alternative splice forms with different pathogen-interaction and inhibition specificities, thus increasing the insect's pattern recognition receptor repertoire. Our previous studies have shown that *An. gambiae* up-regulate splice forms of AgDscam upon *Plasmodium berghei* and *P. falciparum* infections that are specific in the defense against these two pathogens. In order to gain a better understanding of AgDscam-mediated anti-*Plasmodium* defense at the mechanistic level, we performed a yeast-two-hybrid screen using both extracellular and intracellular domains of AgDscam as baits. For the extracellular part, we used the sequence containing the first 8 Ig domains of AgDscam, which showed anti-*Plasmodium* activity in transgenic mosquitoes, as bait and got 7 preys. The intracellular domain turned out to be auto-active for the yeast-two-hybrid assay, therefore we had to cut

it into 3 over-lapping pieces and 2 of them are not auto-active. Using these 2 pieces as baits, we have obtained several preys. We are now in the process of validating the interactions of the proteins with AgDscam and testing their potential functions in anti-*Plasmodium* defense using RNAi-mediated gene silencing.

691

FUNCTIONAL CONFIRMATION OF THE IMPORTANT ROLE OF THE CYTOCHROME P450, CYP6M7 IN PYRETHROID RESISTANCE IN THE MAJOR MALARIA VECTOR *ANOPHELES FUNESTUS*

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Pyrethroid resistance in *Anopheles funestus*, one of the main malaria vectors, is threatening malaria control in Africa. Elucidation of the resistance mechanisms is crucial to implement suitable resistance management strategies. Although progress have been made recently with the detection of CYP6P9a and CYP6P9b resistant genes, the complete set of genes responsible for pyrethroid resistance is still unknown. Taking advantage of recent transcriptome sequencing in An. *funestus*, we designed a new whole genome microarray chip to thoroughly investigate pyrethroid resistance mechanisms in this species. Our work has revealed that besides CYP6P9a and CYP6P9b, another P450 gene, CYP6M7, is playing an important role in pyrethroids resistance mainly in southern African countries. A transcription analysis using microarrays and qRT-PCR, shows that CYP6M7 is highly up-regulated in southern African, especially in Zambia where the CYP6P9a and CYP6P9b over-expression is much lower than in Malawi and Mozambique. Functional characterization of CYP6M7 with an *in vitro* metabolic assays using heterologous recombinant CYP6M7 enzyme in *Escherichia coli* confirmed that this P450 can metabolize both type I (permethrin and bifenthrin) and type II (deltamethrin and lambda-cyhalothrin) pyrethroids commonly used in malaria vector control. Additionally, using transgenic Drosophila melanogaster expressing CYP6M7 through a GAL4/UAS system we have established the role of CYP6M7 in resistance profiles against different insecticides. Furthermore, the analysis of the genetic polymorphism of CYP6P9a, CYP6P9b and CYP6M7 revealed that these genes are under antagonistic selection forces, with the CYP6M7 under balancing selection while CYP6P9a and CYP6P9b are both under directional selection in the three countries. This study suggests that CYP6M7 may have a broader substrate spectrum while CYP6P9a and CYP6P9b have a limited substrate range focusing mainly on pyrethroid insecticides.

693

DIFFERENTIAL INHIBITION OF EFFECTOR CASPASES CASPS7 AND CASPS8 BY INHIBITOR OF APOPTOSIS (IAP1) IN *AEDES AEGYPTI*

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Our laboratory is studying the regulation of apoptosis in *Aedes aegypti*, a vector for several important arboviruses including dengue, yellow fever and Chikungunya, and whether apoptosis can serve as an antiviral defense in mosquitoes during arbovirus infection. Caspases are cysteine proteases that are important in carrying out apoptosis. Six initiator and five effector caspases have been identified in *A. aegypti*, and three of these, including the initiator caspase Dronc and the effector caspases CASPS7 and CASPS8, have been shown to play important roles in apoptosis. Opposing the action of caspases are the negative regulators Inhibitor of Apoptosis 1 (IAP1) and Defense Repressor 1 (Dnr1). Previously obtained RNAi data indicate that IAP1 functions by inhibiting both Dronc and CASPS7, while Dnr1 specifically inhibits CASPS8. However, although IAP1 appears to act specifically through inhibiting CASPS7, binding assays indicated that IAP1 is able to bind to both CASPS7 and 8, albeit more strongly to CASPS7 than CASPS8. Based on these observations, we hypothesized that IAP1 would be a better inhibitor of CASPS7 than CASPS8. To test this, we produced recombinant IAP1, CASPS7 and CASPS8 to use in inhibition assays. We observed that the same concentrations of CASPS7 and CASPS8 had significantly different caspase activities, possibly due to differences in the amount of activated caspase in the two recombinant protein preparations. To normalize the amount of CASPS7 and CASPS8 in our assays, we used active site titration to determine the amount of each active protein. Using same amount of active CASPS7 and CASPS8, we analyzed the ability of IAP1 to inhibit each caspase and found that that IAP1 was 6-fold better at inhibiting CASPS7 than CASPS8. This result corroborates the binding data, and explains why IAP1 acts preferentially through inhibiting CASPS7 during apoptosis.

694

TRANSCRIPTOMICS OF DIFFERENTIAL VECTOR COMPETENCE: WEST NILE VIRUS IN TWO *CULEX* POPULATIONS

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Understanding mechanisms that contribute to viral dissemination in mosquito vectors will contribute to our ability to interfere with the transmission of viral pathogens that impact public health. The expression of genes in two *Culex pipiens quinquefasciatus* populations from Florida with known differences in vector competence to West Nile virus (WNV) were compared using high throughput sequencing. Four day old female mosquitoes from two populations of *Cx. pipiens quinquefasciatus* were fed a blood meal containing 6.0 $\log_{10}$ pfu/ml of West Nile virus. Five days following infection female mosquito bodies were collected and immediately frozen for RNA extraction. Extracted total RNA from each population was sent for transcriptome analysis using Illumina high throughput sequencing. Six RNA-seq libraries were generated from two populations of *Cx. quinquefasciatus*. A total of 15,176 transcripts were combined for comparison of expression differences between the two populations and 118 transcripts were differentially expressed ($p<0.05$). The fold change in expression of the differentially expressed genes ranged from -7.5 - 6.13. The more competent population for WNV (Gainesville) over expressed 77 genes and down regulated 44 genes, compared with the less competent population for WNV (Vero Beach). Preliminary GO function analysis showed that the largest proportion of transcripts was included in the catalytic activity and transporter activity groups except for those in the unknown group. Interestingly, the up- regulated gene set contained most of the catalytic activity function and the down- regulated gene set had a notable proportion of transcripts with transporter activity function. Also, binding and signal transducer activity categories showed different proportions in each up- and down-regulated gene set. Immune response category was shown in only the down regulated gene set, although those represent a relatively small portion of the function. The analysis revealed that the salivary gland genes were over expressed, including gene products involved in odorant binding and blood feeding, in the Gainesville population compared to the Vero Beach population. Validation of the RNA-seq data on a random selection of genes will be discussed.

695

CHARACTERIZATION OF A NOVEL BINDING PARTNER OF THE ANTI-*PLASMODIUM* IMMUNE FACTOR FBN9

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In *Anopheles gambiae* mosquitoes, the IMD pathway is of particular importance as it modulates the development of the human malaria parasite, *Plasmodium falciparum.* We have previously shown that one of the downstream effectors of this pathway, the pattern recognition receptor FBN9, is an antagonist of both bacterial and *Plasmodium* infection. As part of an ongoing effort to identify the exact mechanism that various immune

factors employ to defend *Anopheles gambiae* against pathogens, we used a yeast two hybrid screen to identify novel FBN9 binding partners. Here we provide further characterization of the interaction between FNB9 and GPROP10, a GPCR with no previously identified immune function. Our results show that GPROP10 may also be an *Anopheles gambiae* effector gene. Additionally, biochemical analysis was performed to further analyze this association and to identify the binding region of FBN9 on GPROP10. Since GPCRs are major components of many signal transduction pathways understanding the physiological significance of this interaction can help to further elucidate the role of FBN9 in the mosquito.

696

THE SIGNIFICANCE OF A MOSQUITO HYPER-VARIABLE PATTERN RECOGNITION RECEPTOR, *AGDSCAM*, IN THE MEMORY OF INNATE IMMUNE SYSTEM

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Unlike that of vertebrates the innate immune system of the mosquito appears to lack the adaptive immunity and immunological memory, which relies on the limited numbers of germ line-encoded pattern recognition receptors to generate the specificity towards the pathogen recognition. The studies of the molecular mechanisms that determine the recognition of the pathogens are of the biggest interest. AgDscam, *A. gambiae* down syndrome cell adhesion molecule, which have the potential to generate over 31,000 alternative splice forms with different interaction specificities. We have shown that AgDscam is an essential hypervariable receptor of the *Anopheles gambiae* immune surveillance system, which produces splice form repertoires that are pathogen challenge-specific. Immune priming is a new paradigm in innate immunity. A recent study with the *Anopheles-Plasmodium* system showed that primed mosquitoes previously challenged with parasites developed fewer parasites than control mosquitoes. This result suggests the knowledge gained from immune priming could be used to development new strategies for malaria control. We hypothesized that the alternative splicing of AgDscam plays an important role in immune priming. We started the assay by challenging mosquitoes with 4 different gram positive and gram negative bacteria, and at different time points post infection we collecting hemolymph to assay the expression of AgDscam 101 exons by using CombiMatrix custom microarray. Further detailed studies are undergoing to investigate whether AgDscam are involved in immune priming and memory in the mosquito innate immune system.

697

AEDES AEGYPTI LARVAL PHOTORECEPTORS EXHIBIT PATTERNED EXPRESSION OF RHODOPSINS

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The larval stage of *Aedes aegypti*, the vector for dengue and yellow fever, consists of four larval instars. In all four larval instars, the primary visual system is composed of four ocelli, located on the lateral aspect of the head. The adult compound eye begins to develop during the third instar as a row of individual ommatidial units at the anterior side of the ocelli, and a continuous wave of differentiation then moves in the anterior direction during the third and fourth instar stages to ultimately produce the complete adult eye. We investigated the expression of rhodopsins, G-protein coupled receptors (GPCRs) that initiate visual transduction, in these larval eye structures. Antibodies specific to each rhodopsin were created and used to detect the rhodopsins expressed in the larval retinal structures. The analysis showed that rhodopsins Aaop1, Aaop3, and Aaop7 are each expressed in distinct spatial and temporal patterns. Aaop3 is a major rhodopsin of the larval ocelli photoreceptors. It is localized to photoreceptor cell bodies under light conditions and moves to the photoreceptor rhabdomeres in the dark. Given that the rhabdomeres are the site of phototransduction, the relocation of Aaop3 allows the ocellar photoreceptors to increase light sensitivity under low ambient light conditions. Aaop7 is found in several rows of ommatidia on the anterior edge of the developing compound eye, showing that Aaop7 is expressed only in newly differentiated ommatidial units. Aaop1 was detected later and in the cell bodies of ommatidia within the posterior regions of the developing compound eye. These results reveal that expression of Aaop7 is transient in the developing compound eye and that the expression of Aaop1, the major rhodopsin of the adult R1-6 photoreceptor cells, initiates during these larval stages.

698

ROLES OF FIBRINOGEN-RELATED PROTEIN 1 ON *PLASMODIUM* INVASION IN *ANOPHELES GAMBIAE*

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Malaria is a world-wide health problem that affects two thirds of world population, and causes more than 300 million clinical cases and over 650,000 deaths per year. However, the molecular mechanisms responsible for recognizing malaria parasites in mosquitoes are not yet well understood. Recently, we determined that fibrinogen-related protein 1 (FREP1) is significantly associated with *Plasmodium falciparum* parasite invasion by using high-throughput whole genome sequencing and direct association studies between non-synonymous single nucleotide polymorphisms (SNPs) and *P. falciparum* infection in wild *Anopheles gambiae* mosquitoes from Kenya. Surprisingly, knockdown FREP1 expression by RNAi greatly reduced *P. berghei* infection prevalence and intensity in mosquitoes, while over-expression of FREP1 genes increased *P. berghei* infection in *An. gambiae* mosquitoes. Protein sequence analysis and motifs prediction suggest that FREP1 has a 22-amino acid signal peptide at its N-terminal and the rest portion of protein is extracellular, which was validated by expressing FREP1 in insect cells. The FREP1 protein was secreted from High Five insect cell line into medium. Oligo-array data showed that FREP1 was highly expressed in mosquito midguts. Therefore, we propose that FREP1 acts as a receptor for *Plasmodium* parasites in mosquitoes. To further test this hypothesis, we incubated FREP1 proteins with *Plasmodium* ookinetes, and the results indicated that FREP1 proteins bound *P. berghei* ookinetes very well, which supports FREP1 as a receptor of *Plasmodium* parasites during malaria invasion in mosquito midguts.

699

THE EFFECT OF VIRULENCE FACTORS ON THE PATHOGENICITY OF *STAPHYLOCOCCUS EPIDERMIDIS* IN RATS AND MICE

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Staphylococcus epidermidis is part of the gut microbiota and is the most frequently isolated species of the coagulase negative staphylococci from human stool. However, it is not clear how its presence in the gut affects the cellular structures and functions of this organ. In this study therefore, the pathogenicity of strains of *S. epidermidis* which were isolated from the stool samples of apparently healthy children was investigated in mice and rats. The albino mice (22_30g) and albino rats (100-155g) of both sexes were infected orally and intraperitoneally with graded doses of the bacteria. Acute infection in these animals caused temporary behavioural changes as shown by restlessness and abdominal stretching but did not result in death even at a dosage of 2 x 10^9cfu/ml. Daily administration of the same dose for 14 days resulted in the death of 11 out of 28 (39.3%) mice. Histopathological examination of the affected organs showed congestions, aggregations and multinucleated hepatocytes in the liver, infiltration of the kidney tubule interstitial by chronic inflammatory cells, coagulative necrosis of the kidney, spleen, intestine and stomach cells

as well as marked stroma fibrosis of the spleen. Coagulative necrosis of cells was the most frequently occurring pathological alteration. Lethality and pathological effects reflected the virulence factors expressed by the organism. The results indicate that *S. epidermidis* strains colonising the gut could cause invasive diseases and serious pathological changes in the gastrointestinal tract.

700

HOSPITALIZATIONS AND DEATHS DUE TO DIARRHEAL DISEASE IN CHILDREN UNDER FIVE YEARS OF AGE AT FOUR HOSPITALS IN HAITI, 2010-2012

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Acute gastroenteritis is an important worldwide cause of both hospitalization and death in children under 5 years of age, particularly in low-income countries. In Haiti, where the national introduction of a vaccine against rotavirus, a leading cause of pediatric diarrhea morbidity and mortality, is planned for July 2013, the burden of childhood diarrheal disease is not well understood. We conducted a retrospective review of hospital discharge registries from 2010 to 2012 in the pediatric wards of four hospitals_two in Port-au-Prince, and one each in Artibonite and Southeast Departments_that are part of a laboratory-enhanced syndromic surveillance system in Haiti. We recorded the number of all-cause and diarrhea hospitalizations and deaths by age (<2 and 2-5 years of age) and epidemiological week. A diarrhea hospitalization was defined as one due to diarrhea, acute gastroenteritis, dehydration, or intestinal parasitosis. Of 10,621 total hospitalizations in children under 5, 3,582 (34%) were for diarrhea, including 665 (27% of total hospitalizations), 1,117 (33%), and 1,800 (38%) in 2010, 2011, and 2012, respectively. Eighty-nine percent (3,169/3,582) of pediatric diarrhea hospitalizations were in children under 2. Among 540 deaths in children under 5, 62 (11.5%) were due to diarrhea, and 60 of the 62 diarrheal deaths were in children under 2. The case fatality rate among hospitalized diarrhea patients under 5 was 1.7%. There appeared to be two seasonal peaks in diarrhea hospitalization - a taller February-May peak and a smaller peak in October. From 2010 to 2012, diarrhea was a major cause of hospitalization and death in children under 5 in four hospitals in Haiti; the greatest burden was among children less than 2. The annual increase in the proportion of diarrheal patients among all hospitalized patients during this period may be partly explained by the cholera epidemic, which began in October 2010. Continued hospital-based surveillance of pediatric diarrhea hospitalizations and deaths will enable assessment of the impact of rotavirus vaccine introduction in Haiti.

701

IMMUNOGENICITY OF AN ORAL CHOLERA VACCINE, SHANCHOL, IN A LARGE FEASIBILITY STUDY IN BANGLADESH

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Bangladesh is an endemic region for cholera as a result prevention and control of this disease is important using immunoprophylactic measures with vaccines. Immunization against cholera is considered a suitable public health tool for preventive measures since it is difficult to provide safe drinking water and proper hygiene in the near future to high risk areas prone to cholera. Therefore, a study was conducted to ascertain the immunogenicity of cholera vaccine, Shanchol within a large feasibility study in Bangladesh which was conducted in a high risk population in Mirpur in urban Dhaka. From a total of over 141,000 participants, a subgroup of 330 people were from the six study wards of Mirpur (2,4,5,6,14 and 16). The objective was to measure the immunogenicity of the cholera vaccine, Shanchol when administered in a large mass immunization program. The subgroup comprised of adults (18-45 yr: n=110), toddlers (2-5 yr: n-110) and younger children (12-23 mo: n=110). The two-dose regimen of the vaccine was administered orally at least 14 days apart, no serious adverse events was elicited or recorded for any participant throughout the study. Vibriocidal antibody responses in adults were 79% to V. cholerae O1 Inaba, 81% to V. cholerae O1 Ogawa. In the toddlers responses were 87% and 90% to O1 Inaba and Ogawa respectively. In the youngest age group it was 76% and 74% to Inaba and Ogawa respectively. The responses in all ages were higher at day 7 and day 21 compared to pre-immune titers (P<0.001). Overall the antibody response was 81% in all age groups to V. cholerae O1 Inaba and Ogawa. Similar immunogenicity profiles were seen in a previous pilot study which was conducted before initiating the evaluation of large scale feasibility study in Bangladesh. The result of this study is very encouraging, showing that the batch of Shanchol vaccine used was safe as well as immunogenic, giving robust antibody responses in all age groups of individuals. The study thus shows that there is benefit in use of the oral cholera vaccine using the existing national immunization system to target all age groups above 1 year of age in the future in cholera endemic countries like Bangladesh living in high risk conditions.

702

VIBRIO CHOLERAE OUTBREAK IN BATALA, PUNJAB, INDIA - 2012

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In India, acute gastroenteritis (AGE) is a major public health problem; in Punjab state, 15 cholera outbreaks out of a total of 39 AGE outbreaks were reported in 2012. In October 2012, a death due to AGE was reported at Civil Hospital Batala, followed by a sudden rise in the number of hospitalized AGE cases. We sought to establish the cause and source of infection. A case was defined as a person who had three or more loose stools per day between 25th September to 10th November 2012 from Gandhi Nagar camp and adjoining areas. To ascertain cases, we conducted a house to house survey in the affected area. Water specimens were tested using the method of "Most Probable Number (MPN)" for potability of water; culture of stool samples was performed to identify the causative agent. Epi-info & MS excel were used for analysis. A total of 834 cases and 33 deaths were identified from population of 24,765 (attack rate (AR): 3.4%, case fatality ratio: 4%). The AR was significantly higher among females (n=440) than males (n=394) (3.7% vs. 3.0%, p=0.002) and among those older than 55 years (n=105) vs. under/equal to 55 years (n=729) (4.8% vs. 3.2%, p<0.001). The most affected area was Gandhi-Nagar Camp (AR=6.6%) followed by Guru Nanak Nagar (AR=2.1%) and Murgi-Mohalla (AR=0.60%). *Vibrio Cholerae* O1 Ogawa was identified in 11 out of 35 stool samples; 12 water samples obtained from 23 households demonstrated fecal contamination. Bacterial growth and contamination from sewage were identified in piped drinking water which served these homes.The piped water supply to the affected area was immediately stopped and an alternate source of water supply (tanker) was arranged; reports of new cases declined. In conclusion, sewage-contaminated water was likely the source of infection. Chlorine tablets and ORS packets were distributed with health education in the community. Prompt public health action through identification of the source of contamination and the implementation of control measures stopped the occurrence of new cases thereby limiting the scale of the outbreak.

703

EPIDEMIOLOGY OF NOROVIRUS IN PUERTO MALDONADO, PERU

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Norovirus is the leading cause of acute gastroenteritis worldwide. Direct measurement of community incidence of norovirus gastroenteritis is largely limited to developed country settings. In October 2012 we implemented a prospective population-based cohort study of diarrheal disease comprised of 265 households and 1350 persons in Puerto Maldonado, in the Southern Amazon basin of Peru. Households were visited three times per week and stool samples were collected from persons who experienced diarrhea (defined as three or more loose or liquid stools in any 24 hour period). Samples were tested using real time-reverse transcription polymerase chain reaction (real time RT-PCR). From October 15, 2012 to April 2, 2013, 208 cases of diarrhea were identified (33.8 episodes/100 person-years of follow-up). Samples were obtained from 56.3% (117/208) of persons with diarrhea, of which 16.2% (19/117) were positive for norovirus _ 5.1% (6/117) for genotype I and 11.1% (13/117) for genotype II. The median age of norovirus positive individuals was 2.6 years old (IQR 21.6; SD 24.3). Vomiting was also reported in 36.8% (7/19) of those norovirus positive. Three persons (0.2%) required outpatient care and one person (0.1%) required hospitalization. Based on the assumption that individuals who gave a specimen were representative of the etiology of all cases, norovirus incidence was calculated to be 5.4 episodes per 100 person years. Norovirus, predominantly genogroup II, is a common cause of diarrhea in the area of study. Longitudinal data collection from this cohort will allow us to further understand the economic burden, seasonality, and risk factors for intra-household transmission of norovirus in Peru.

704

THE INDIAN OCEAN DIPOLE AND CHOLERA INCIDENCE IN BANGLADESH

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It has been reported that El Niño-Southern Oscillation (ENSO) plays a role in the interannual variation of endemic cholera in Bangladesh. The Indian Ocean Dipole (IOD) is also associated with interannual climate variability in the tropical Indian Ocean. We explored the relationship between the IOD and the incidence of cholera in Bangladesh. A generalized linear negative binomial model was used for time-series regression of the number of monthly hospital visits for cholera in Dhaka and Matlab and the Dipole Mode Index (DMI), controlled for ENSO index (NINO3) and seasonal and interannual variations. We also performed a cross wavelet coherency analysis to examine whether the association between the IOD and the incidence of cholera was stationary (i.e., constant through time). From the generalized linear model, the increased number of cholera cases in Dhaka was associated with a higher DMI at a lag of 0-3 months, while it was also associated with lower DMI at a lag of 4-7 months. In Matlab, increased number of cholera cases was associated with a higher DMI at a lag of 0-3 months and with high NINO3 at a lag of 8-11 months. The increased risk of hospital visits for cholera was associated with high SSH and SST in both areas. Cross wavelet coherency analysis revealed that the strength of both the IOD and ENSO associations with cholera hospitalizations changed across time scales during the study period. In Dhaka, 4-year long coherent cycles were observed between cholera and the index of IOD in 1988-1997. In Matlab, the effect of ENSO was more dominant while there was no evidence for an IOD effect on cholera hospitalizations. Our findings support a hypothesis that a negative and positive dipole event may increase potential flooding and thus an outbreak of cholera in Bangladesh in different lag time whilst the association was time-varying.

705

CLOSTRIDIUM DIFFICILE: AN EMERGING ZOONOSIS?

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Clostridium difficile is an anaerobic bacillus that can be extremely pathogenic. This infection occurs in both humans and animals such as canines, equines, and bovines. Human infections typically are nosocomial, however, investigators recently have noted an emerging trend of community-acquired cases. Although no confirmed cases of foodborne *C. difficile* have been reported, the pathogen has been found in retail meat products. Moreover, it is a common pathogen in domesticated animals such as canines. This review examines potential risks for community-acquired *C. difficile* and explores further research in this area.

706

EVALUATION OF RISK FACTORS AND CARRIAGE OF ENTERIC PATHOGENS IN CHILDHOOD DIARRHEA IN FOUR RURAL COMMUNITIES IN HAINAN, CHINA

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China's Ministry of Health, with UNICEF, WHO, and UNFPA, found disparities in child mortality between urban and rural areas of China. Rural areas accounted for over 70% of childhood deaths of which 18% were from Diarrhea. This study focused on the determinants and characteristics of diarrheal disease in children under five years old in four communities of Hainan, China. A survey was completed by the primary caregiver of 413 randomly selected children and a stool specimen was collected. BioFire Diagnostics, Inc. analyzed 105 stool samples using a Film Array (multiplexed PCR device) GI panel for detection of 23 enteric pathogens. Water samples were collected from each family's water source and analyzed for *coli*form forming units (CFU) and *E. coli*. Survey results showed 23% of children had diarrhea in the previous 2 weeks and BioFire testing identified an average of 3.6 pathogens per child. Fecal specimens were positive for the following 18 pathogens: EAEC 73%, ETEC 71%, EPEC 76%, STEC 19%, *Campylobacter* 15%, *Shigella*/EIEC 16%, *Aeromonas* 4%, *P. shigelloides* 6%, *C. difficile* 4%, *Salmonella* 3%, *V. cholera* 1%, *G. lamblia* 28%, *Cryptosporidium* 9%, *Adenovirus* 6%, *Norovirus* 5%, Human *Astrovirus* 2%, *Sapovirus* 2%, and *Rotavirus* 1%. Water in all communities was contaminated with fecal flora. The average MPN of fecal flora/100ml of water was: unprotected well water 31,067; public tap/standpipe 25,330; protected well 19,906; tubewell/borehole 5,244; piped into dwelling 3,706; bottled water 925; and piped water 732. Decreased diarrhea correlated with soap observed in household, use of soap in past 24 hours, piped water source and utilization of toilet facilities. Education of mother and proximity of wells to animals or toilet facilities did not influence prevalence of diarrhea. The number of enteric pathogens per child was not influenced by the risk factors studied. With multiple pathogens per child it is difficult to determine the etiological cause of diarrhea. Presence of *Cryptosporidium, Shigella, Campylobacter* and *Giardia* was associated with increased incidence of diarrhea. The number of enteric pathogens per child suggests that these children live in a highly contaminated environment and may be vectors of gastrointestinal disease. This data will be discussed with health care providers and study participants to generate strategies to reduce childhood diarrhea.

707

MODELING CHOLERA IN A PATCHY ENVIRONMENT WITH WATER AND HUMAN MOVEMENT

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The World Health Organization estimates that there are 3m-5m cholera cases per year with 100,000 deaths across 40-50 countries. For example, there has been a recent cholera outbreak in Haiti. Cholera is a waterborne bacterial disease caused by the bacterium "*Vibrio cholerae*", which is an aquatic organism. The movement of both humans and water have recently been suggested to influence the spatial spread of cholera in Haiti. To better understand this spatial spread, a new compartmental cholera model is formulated that incorporates patch structure, and both water and human movement. The water and human movement connect individual patches (communities), resulting mathematically a weighted directed graph (a community network). When can a disease like cholera invade this network? Our mathematical results show analytically that the answer to this question depends on both the network structure as well as on the properties of the individual patches. In some situations, the basic reproduction number, which determines the invasibility of cholera on the community network, becomes a weighted average of the patch reproduction numbers, with weights given by the network structure in terms of the net inflow. That is, patches with the most net inflow have the greatest impact on invasibility. Our results also show that clustering disease hot spots together with respect to meta-communities increases the ability of the disease to invade.

708

FREQUENCY OF SERINE PROTEASE AUTOTRANSPORTER PROTEINS *ENTEROBACTERIACEAE* (SPATE) IN *ESCHERICHIA COLI* DIFFUSELY ADHERENT (DAEC) ISOLATED FROM CHILDREN WITH AND WITHOUT DIARRHEA

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Diffusely adherent *Escherichia coli* (DAEC) strains have been recognized as the sixth class of diarrheagenic *E. coli* (DEC) and appear as a heterogeneous group. Previous studies have shown the frequency of SPATE genes as virulence factors in the DEC however have not been identified in strains DAEC. The aim of this study was to analyze the frequency of 6 SPATE encoding genes in DAEC isolates from children with and without diarrhea. We have analyzed 104 isolates from children with diarrhea (63) and no diarrhea (41). Two conventional multiplex PCR were used to identify the presence of six virulence genes belonging to the SPATE group (*sigA, pet, espP)* and *(sat, pic,espC*). The EAEC O42 (*pet, pic*), EHEC 933 (*espP*), EPEC 2348/64 (*espC*) and *Shigella flexneri* 1106 (*sigA, sat*) were used as positive controls. *sat* gene was identified as the most prevalent in both cases 40% (25/63) and controls 42% (17/41), followed by *espP* with a frequency of 21% (13/63) for cases and 10% (4/41) for controls, *pet* 8% (5/63) for diarrheal cases and 5% (2/41) in controls, *sigA* 8% (5/63) in diarrhea and 12% (5/41) in controls; *espC* and pic 5% (2/41) only in controls. With these results we conclude that DAEC strains may possess SPATE-encoding genes irrespectiveness if they are isolated in patients with diarrhea or healthy children.

709

PREVALENCE OF CAPSULAR TYPES OF *CAMPYLOBACTER JEJUNI* ISOLATES FROM SYMPTOMATIC AND ASYMPTOMATIC CHILDREN IN THE PERUVIAN AMAZON

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Campylobacter jejuni is one of the leading causes of diarrhea worldwide and the development of a protective vaccine is critical. Capsular polysaccharides (CPS) conjugate vaccines have been shown to be protective against *C. jejuni* infections in non-human primates. Continued pursuit of such a vaccine strategy will require answering questions about the required valency of a broadly effective CPS conjugate vaccine against *C. jejuni*. Of importance to this effort will be determining the prevalence of CPS types circulating globally. As a step in that direction, a multiplex PCR assay for detection of CPS encoding genes capable of distinguishing 24 of the 47 CPS types was performed on *Campylobacter* isolates from a longitudinal case-control study conducted between 2002 and 2006 in a cohort of children under 72 months of age, located in a rural community in Iquitos, Peru. This study included 131 symptomatic and asymptomatic individuals of which 206 *Campylobacter jejuni* isolates were acquired from stool samples. Multiplex PCR results demonstrated that the most common CPS types were HS15 (17%), HS8/HS17 (14%) and HS3 complex (12%), while 7% of isolates were non-typeable. Differences in CPS type distribution between symptomatic and asymptomatic infections for HS15, HS8/HS17 and HS3 complex were not statistically significant, while HS2 was detected in symptomatic cases only. Most subjects (39 out of 45) had multiple infections of which each were with an isolate of a different CPS type, suggesting that acquired immunity may be protective. This data represents the first report examining the distribution of CPS types in South America.

710

PREVALENCE OF BACTERIAL ENTEROPATHOGENS AND THEIR ANTIBIOTIC RESISTANT PROFILES IN STOOL SAMPLES FROM CHILDREN IN THE PERUVIAN AMAZON

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Diarrheal diseases have a substantial impact on childhood development and mortality in developing countries. Our study was conducted to determine the most common enteropathogenic bacteria in children with in the small Amazon town of Santa Clara just outside of Iquitos-Peru. During this study, 203 children under 5 years of age were monitored bi-weekly for 7 months with asymptomatic stool samples taken monthly and diarrheal stool samples taken when sick. Fecal samples from both symptomatic and asymptomatic participants were cultured on standard media with antibiotic susceptibility determined by disk diffusion. A total of 1925 fecal samples were cultured, from which 500 represented diarrheas. Of the samples tested 12.78% (246/1925) were positive for enteropathogenic bacteria, representing 13.8% (69/500) of the diarrheal samples and 12.42% (177/1425) of the non-diarrhea samples (p=0.4). In total, 257 bacteria were isolated (235 samples with a single pathogen and 11 samples with 2 pathogens isolated). *Campylobacter* (63.6% *C. jejuni* and 36.4% *C. coli*) was the most common enteropathogen present in 72.76% of the isolates, followed by *Shigella* (16.34%), *Plesiomonas shigelloides* (8.17%), *Aeromonas hydrophila* (1.17%), *Salmonella* (1.17%), and one isolate of *Vibrio cholera* Non O1 (0.39%). Of the *Campylobacter* isolates, 81.28% were resistant to ciprofloxacin,67.38% to trimethoprim/sulfamethoxazole,

44.39% to ampicillin, 41.18% to tetracycline, and 11.76% to azithromycin. Among *Shigella* isolates, resistance to tetracycline was 97.62%, ampicillin (80.94%), trimethoprim/sulfamethoxazole (78.57%), azithromycin (11.90%) and ciprofloxacin (0.00%). Our study showed that *Campylobacter* was the most common enteropathogen among both symptomatic and asymptomatic children and was found to be resistant to multiple antibiotics. Our data suggests that children can also be asymptomatic carriers of *Campylobacter*, which would be a significant public health problem in developing countries.

711

PATTERNS OF BACTERIAL ECOLOGY AND ANTIBIOTIC RESISTANCE IN TWO REGIONS OF PERU, 2007-2009

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Antibiotic use in Peru lacks regulation, therefore the effective treatment of diarrheal disease is often complicated by antibiotic resistant organisms. In this study, we investigated the patterns of antibiotic resistance in coastal and jungle regions of Peru. From 2007-2009, fecal samples were collected from healthy children under 5 years living in the Ica province, located on the coast, and the Alto Amazonas province, located in the Amazon rainforest. Samples were plated on MacConkey agar for the detection of lactose fermentation, TCBS for *Vibrio*, charcoal agar for *Campylobacter*, and Hektoen agar for *Shigella* and *Salmonella*. PCR was performed for *E. coli* typing and bacterial isolates were tested for antibiotic resistance by disk diffusion. Pathogenic bacteria were isolated from 11% (80/744) of the samples cultured. Of 76 samples from which a single pathogen was isolated, the type of bacteria differed between the coast and the jungle ($p<0.001$). Ninety percent (43/48) of single-pathogen isolates from the jungle were identified as enterotoxigenic *E. coli*; of the coastal isolates, 36% (10/28) were identified as *Campylobacter jejuni*, 36% (10/28) as *Aeromonas,* and 0% as enterotoxigenic *E. coli.* Overall, 99% of single-pathogen isolates demonstrated resistance to at least one antibiotic, with 77% demonstrating resistance to ampicillin, 58% to erythromycin, 51% to each of cotrimoxazole and cephalotin, and 49% to tetracycline. Fifty-two percent of all single-pathogen isolates were resistant to more than one antibiotic. Isolates from the jungle displayed greater resistance to erythromycin (81% vs. 21%), cotrimoxazole (63% vs. 8%), and tetracycline (56% vs. 23%; all ps<0.04), while resistance to ciprofloxacin was greater on the coast (55 vs. 6%; $p<0.001$). These findings suggest that some degree of antibiotic resistance is nearly universal among bacteria isolated from the Peruvian jungle and coast, and that regional differences in resistance patterns should be considered in the treatment of enteric disease in Peru.

712

HUMAN DIARRHEA INFECTIONS ASSOCIATED WITH DOMESTIC ANIMAL HUSBANDRY: A SYSTEMATIC REVIEW

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The majority of infectious diseases that affect humans have a zoonotic origin. While zoonotic diseases are commonly discussed from the perspective of emerging infectious diseases, certain enteric pathogens that affect humans have animal reservoirs. Available data on the relationship between enteric pathogens in animals and diarrheal illness in humans has not been examined systematically in the context of domestic animal husbandry. Further exploration of the risk that domestic poultry and livestock pose to human health is required to identify hazards and mitigation strategies. We conducted a systematic review, and meta-analysis if possible, to determine the impact of domestic husbandry of poultry and livestock on diarrheal disease in humans. PubMed/Embase/ISI Web of Science were searched until February 25, 2013 without restrictions on language or year or publication. Bibliographies of selected articles were searched. Eighteen studies met our eligibility criteria. All types of studies with data on presence of domestic animals (poultry, ruminants, goats and swine) and diarrhea in humans were considered. Odds ratios reporting association between domestic animal exposure on diarrheal illness were extracted from the literature or calculated using provided data. Quality of each study was assessed using grading criteria set by the authors. All studies were divided among 18 animal exposure-disease strata; only one stratum containing a sufficient number of studies to perform a random effects meta-analysis. Domestic exposure to poultry was significantly associated with human campylobacteriosis (OR 2.49, 95% CI 1.63-3.81). In addition, 14/19 studies included in this review reported a positive association between domestic animal exposure and diarrheal illness. In conclusion, our results indicate domestic poultry and livestock exposure is associated with increased risk of diarrheal illness in humans. There was a potential for biases, both within our study (publication bias) and between our studies (principally recall bias). There was also considerable heterogeneity of effect among the studies included for meta-analysis. Despite these limitations, we found evidence that domestic animal exposure should be considered a risk factor for human diarrheal illness. Further study may confirm and clarify this relationship and interventions, such as corralling and pasturing, should be explored.

713

BACTERIAL AND PARASITIC ETIOLOGIES OF DIARRHEAL DISEASE IN THE PERUVIAN AMAZON, 2003-2011

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Diarrheal disease is a major threat to military populations and has a high prevalence in the Amazonian region of Peru. From 2003 to 2011, diarrheal disease surveillance was conducted among personnel at the Vargas-Guerra Army recruit training base in Iquitos, Peru. All asymptomatic individuals newly stationed at the base were invited to participate in providing a baseline stool sample, and all subjects experiencing diarrhea during their time on the base were requested to present to the Army health post where a stool sample was taken for stool culture and ova and protozoan parasitic detection by microscopy. We conducted a case-control analysis of these data to identify the bacterial and parasitic etiologies of diarrhea in this population. Of 644 diarrhea cases who provided stool samples, 522 (81.0%) could be matched by calendar month with appropriate control baseline samples (which were provided after at least 7 days on the base). *Cryptosporidium* (24.5%), *Giardia lamblia* (18.9%), and *E. histolytic* (16.4%) were the most frequently detected pathogens in samples. *Shigella flexneri Enterotoxigenic E. coli* (ETEC) *and Campylobacter sp.* were detected in 10.3%, 7.1% and 2.0% of samples, respectively. Diarrhea case status was associated with the detection of *Shigella flexneri* (Odds Ratio (OR)=5.71, 95% CI=3.39, 9.63), ETEC (OR=3.02, 95% CI=1.76, 5.18), and *Cryptosporidium* (OR=1.57, 95% CI=1.18, 2.09). No other pathogens assessed were significantly associated with the risk of diarrhea. These data demonstrate the diversity of the etiology on diarrheal diseases in a military population in the Amazon and the importance of *Shigella flexneri*, *Enterotoxigenic E. coli* and *Cryptosporidium* as diarrheagenic agents in this population, which highlight the need for measures to prevent infection and transmission of these pathogens in the region.

714

EVALUATION OF *CLOSTRIDIUM DIFFICILE* TREATMENT WITH FECAL MICROBIOTA TRANSPLANTATION

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Clostridium difficile is a gram positive spore-forming anaerobic bacterium that is responsible for causing diarrheal disease around the globe. This bacterium is found in increasing prevalence worldwide causing rising rates of infection. *C. difficile* infection (CDI) is reported primarily as a nosocomial disease and is estimated to cost the U.S. over $3.2 billion dollars a year in healthcare. CDIs are becoming more common, serious, difficult to treat, and more likely to recur. The CDC states that CDI claims 14,000 American lives each year. Current treatment for CDI involves high-powered antibiotic therapy. *C. difficile* strains are becoming more resilient and less effected by antibiotic treatment, especially in relapsing cases. This review was completed in order to examine a treatment option that may prove to be beneficial in the fight to cure people with chronic CDIs. The purpose of this review is to examine fecal microbiota transplantation (FMT) as a potential treatment for CDIs. FMT needs to be further studied as a possible future treatment of CDIs. The following key words were used in order to conduct a thorough review of the pertinent literature: *C. difficile* infection (CDI), fecal transplants, and *C. difficile*. Articles were primarily found through PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/). General guidelines for CDI treatment was discussed in the literature and then compared to the treatment with FMT. The methodology and success rates of the various treatments for CDI were also examined. Evidence from multiple case reports and case studies revealed high success rates for treatment of CDI with FMT. Results of the first randomized clinical trial (RCT) also supports better patient outcome with FMT therapy in people with relapsing CDI. In-fact, results using FMT therapy had significantly higher efficacy for the treatment of recurrent CDI than the use of vancomycin; which is the standard antibiotic used to treat CDIs. Conventional antibiotic therapy eliminates the normal flora of the GI tract, making optimal conditions for *C. difficile* to flourish. FMT helps recolonize the GI tract of the natural flora using donor feces. Fecal transplantation reveals a hopeful outlook as a potential future cure for CDIs, as well as a possible treatment option for a various number of bowel maladies. This opens the door for future research to learn of the potential benefits using FMT for a wide array of conditions.

715

ESTIMATING THE FORCE OF INFECTION OF ENTERIC PATHOGENS

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Diarrheal disease remains the second highest cause of childhood mortality despite knowledge of, and improvements to, interventions. A better understanding of the mechanisms that result in exposure to and infection from specific enteric pathogens is necessary to better target interventions and measure their impact. A key mechanism is immunity. The incidence of diarrheal episodes is frequently reported to decrease as children age and observational studies have inferred that this phenomenon indicates the acquisition of immunity that reduces the incidence of infection. Additionally, incidence of diarrhea in breast-fed children is lower than in weaned/not breast-fed children of the same age. Here we present an analysis of empirical age-prevalence data from MAL-ED, a longitudinal study of enteric disease in children from 8 populations. For each of a number of common enteric pathogens we apportions the risk of pathogen presence into the probabilities of population-specific exposure to enteric pathogens, the probability of infection as a function of age and the probability of re-infection reflecting previous exposures. We contrast the difference in the quantitative combination of these three components between pathogens and discuss how knowledge of these biological mechanisms informs possible intervention options.

716

NEUTROPHILS EXHIBIT JANUS-LIKE BEHAVIOR DURING *ORIENTIA TSUTSUGAMUSHI* INFECTION IN A MURINE MODEL OF SCRUB TYPHUS

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Scrub typhus is a seriously neglected disease with approximately one-third of the world's population at risk of being infected with *Orientia tsutsugamushi*, and the occurrence of over one million scrub typhus cases annually illustrate its importance in global health. *S*crub typhus is caused by a rickettsia transmitted by the parasitic larval stage of trombiculid mites, primarily of the genus *Leptotrombidium*. All scrub typhus case studies that report blood cell counts, describe neutrophilia during the course of infection. Patients with confirmed scrub typhus have significant increases in activated neutrophil proteins in serum, and the increase of neutrophil recruiting cytokines. We also observed neutrophilia in intravenously infected mice, suggesting key role for neutrophils in scrub typhus disease progression. To determine the role of neutrophils in this infection, female C57BL/6 mice were lethally challenged, and neutrophils were depleted one day prior to infection (D-1), one (D+1), six (D+6), or one and six (D+1/6) days post infection. The effects of neutrophil depletion were observed to be dependent on the time post infection. Animals depleted early (D-1 and D+1) or twice post infection exhibited more severe pathology at an earlier time point and had disease progression similar to non-depleted animals but had greater survival and lower bacterial loads in the organs. Animals depleted 6 dpi recovered weight, and signs of illness had resolved by 12 dpi. Histopathology demonstrated decreased cellular infiltrates when compared to infected, non-depleted animals. Depletion of neutrophils decreased mortality independent of when depleted, but only depletion 6 dpi resulted in amelioration of signs and early weight recovery. These data suggest a dual role of neutropils in bacterial clearance and tissue pathology during scrub typhus infection.

717

INJECTIONAL ANTHRAX: AN EMERGING PUBLIC HEALTH ISSUE

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Bacillus anthracis is the pathogen that causes anthrax. Historically, clinicians have classified into three types: cutaneous, gastrointestinal, and inhalational. More recently, however, a fourth form, injectional anthrax, has emerged among heroin users in Europe. First identified in 2000 in a single intravenous drug user, additional cases of injectional anthrax soon were reported throughout Europe over the following decade. Transmission occurs when intravenous heroin, surreptitiously contaminated with *B. anthracis*, is injected into a vein of the addict. The purpose of this study is to review the existing literature on injectional anthrax and to assess the significance of its emergence for physicians and public health officials. The following key words were used to conduct an extensive review of the pertinent literature: Bacillus anthracis, anthrax, heroin, injectional, drug use. Once identified, each article was analyzed for descriptors of patients (age, sex, drug use, nationality), signs/symptoms, outcome (death or recovery), and advice to physicians, if present. Due to a lack of identifiers among the patients/cases, it is difficult to determine their exact numbers. As of early 2013, over 130 different suspected cases were reported, the majority of which (n =119) occurred in Scotland. All patients

identified were intravenous drug users who have used heroin within the previous 1-4 days. Symptoms ranged from mild cutaneous lesions to septic shock, with the majority presenting with sever soft tissue infections at the injection site. Although the mortality rate improved through the outbreak as physicians became more aware of the infections and followed more cautious protocol, mortality still remained around 30%. As 90% of the world's heroin originates in Afghanistan, an area where anthrax is endemic, clinicians and public health officials should be increasingly aware of this novel form of anthrax.

718

EPIDEMIOLOGY OF Q FEVER IN THAILAND

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Q fever is zoonotic disease caused by the bacterium *Coxiella burnetii*. This bacterium has been identified as a cause of endocarditis in Thailand, but data on animal reservoirs and frequency of human infection are lacking. Three different human and animal populations were screened in an attempt to determine the prevalence *C. burnetii* antibody and identify animal reservoirs of the disease in Thailand. All sera were tested by commercial ELISA kits for the presence of *C. burnetii* IgG antibody. Human ELISA positive sera were confirmed and titrated by Indirect Fluorescent Antibody assay using phase 2 antigen. Samples with IFA titer $\geq$ 64 were considered positive for *C. burnetii* IgG antibody. First, patients hospitalized with prolonged fever of unknown origin at an academic hospital in Bangkok and participating in a rickettsial disease study were included in the investigation. Acute serum samples from 28 of 152 patients (18%) were IgG positive, indicating evidence of *C. burnetti* exposure, while convalescent testing is pending. In the second screening, a single serum samples were collected from dairy cattle, goats and their owners from farming communities in Chiangmai, Nakonratchasima and Nakonsithammarat provinces of Thailand from January 2012 through June 2013. Cattle showed 4% (28/780), and goats 6% (17/300) positivity for *C. burnetii* antibody. Twenty-seven of 56 livestock farmers' sera (48%) were positive for *C. burnetii* IgG antibody. In the third population, captive wildlife and their caretakers in Chiangmai zoos were screened in May 2012. Of 61 captive wild deer and ruminant species, none were positive, while 12 of 104 wildlife caretakers' sera (12%) were positive for *C. burnetii* IgG. These results demonstrated the high prevalence of *C. burnetii* antibodies among persons with prolonged fever and those with animal exposure and identified likely animal reservoir. More extensive investigations are needed to determine disease risk factors and to describe the clinical presentation and incidence of acute human infection.

719

RECOMBINASE POLYMERASE AMPLIFICATION ASSAY FOR RAPID DETECTION OF Q FEVER

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Q fever is a worldwide zoonotic infection caused by the intracellular bacterium *Coxiella burnetti*. Transmission generally occurs via inhalation of aerosolized bacteria in air that is contaminated with infected animal tissues. The clinical syndrome of illness caused by *Coxiella burnetti* is often non-specific which can make diagnosis challenging. In the current diagnostic scheme comparison of serologic samples in both the acute and convalescent stages of infection in conjunction with a clinical syndrome that is consistent with Q fever are the diagnostic standards. Polymerase chain reaction techniques have been developed which are rapid, sensitive and qualitative for the detection and diagnosis of acute (< 2 weeks of symptoms) Q fever. To date there have been no assays developed for rapid and accurate point of care or field testing for Q fever. Recombinase polymerase amplification (RPA) is a novel technology for the amplification and detection of DNA or RNA. RPA is an isothermal amplification process that uses the combined properties of the bacterial recominase (RecA) polymerase and exonuclease**,** to achieve the amplification of specific DNA sequences. The advantage of RPA is that amplification can be done rapidly (< 20 minutes) without the need for expensive thermocycling equipment. Product can be detected with the use of a simple sandwich assay which also eliminates the need for additional detection instruments. The goal of this project was to adapt the RPA assay for detection of Q fever targeting the *IS1111a* transposase gene which is present in multiple copies in the *Coxiella burnetti* genome. Several sets of unique primer sequences were designed for the *IS1111a* transposase gene and the reactions were carried out at 37°C for 20 minutes. The performance of many different combinations of forward and reversed primers was evaluated. The sequences of the probes were designed for the two best primer sets. Endpoint detection of amplicon has been achieved using the TwistAmp nfo kit (*TwistDx*) in conjunction with the Type II Best Cassette (*Biohelix*). This assay showed a detection limit of 25 copies per reaction. To our knowledge our assay is the first developed using RPA for the molecular detection of Q fever. The speed and portability of this assay could be useful in both point of care and field diagnostics for acute Q fever infection.

720

CHARACTERIZATION OF CLASS 1 INTEGRONS IN *ESCHERICHIA COLI* ISOLATES FROM BLOOD ORIGIN IN PERUVIAN CHILDREN

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Escherichia coli is the species that most frequently causes gram-negative *bacteremia*. Resistance of gram-negative organisms to antibiotics such as β-lactams, aminoglycosides, trimethoprim and chloramphenicol is caused by many different acquired genes, and a substantial proportion of these are part of small mobile elements known as gene cassettes. Gene cassettes can move into or out of a specific site *(attI* site) in a companion element called as integron, and integration or excision of the cassettes is catalyzed by a site-specific recombinase (IntI) that is encoded by the integron. Integrons are genetic structures able to capture, excise and express genes, frequently included in mobile elements such as plasmids that allow their dissemination among bacteria. This study presents the antibiotic susceptibility and the prevalence and characterization of Class 1 integrons in clinical *E. coli* isolates from blood. Antibiotic resistance was analyzed by the method of Kirby Bauer and the presence of class 1 integrons was determined by PCR in 64 *E. coli* causing *bacteremia* in hospitalized children younger than 5 year of age. To determine the composition of variable regions, amplified products were digested with *HinfI,* and resolved in 2% agarose gels. Representative samples from each RFLP pattern were purified and sequenced. *E. coli* from blood exhibited high levels of antimicrobial drug resistance in blood ampicillin (91%), cotrimoxazole (67%), tetracycline (52%), and gentamicin (44%). Integrons type 1 were found in 22 (31%) isolates, in which five different integrons were detected. In total different seven genes cassettes were found (*aadA1, aadA2, aadA5*) encoding enzymes that confers resistance to aminoglycosides and (*dfrA7, dfrA12*, *dfrA15, dfrA17*) conferring resistance to trimethoprim. These findings indicate that integrons of class 1 play an important role in resistance to trimethoprim and aminoglycosides in children less than five years.

721

EVALUATION OF THE PATHOGENIC POTENTIAL OF *RICKETTSIA AMBLYOMMII* IN GUINEA PIGS (*CAVIA PORCELLUS*) AND PROTECTIVE IMMUNITY AGAINST *R. RICKETTSII*

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Rickettsia amblyommii belongs to the spotted fever group (SFG), which includes several vector-borne human and animal pathogens. Although *R. amblyommii* was determined non-pathogenic in one experimental animal model, there is serologic evidence of possible infection and mild disease in humans. Also, earlier experiments have shown protective immunity against R. rickettsii in guinea pigs that have been previously infected with SFG *Rickettsia*, but this has not been investigated with *R. amblyommii*. The aim of this study was to evaluate the pathogenic potential of *R. amblyommii* in guinea pigs and its capacity to generate protective immunity against *R. rickettsii*. Six guinea pigs were inoculated intraperitoneally with *R. amblyommii* and 2 controls with culture medium. Necropsies were performed in duplicate on infected animals at days 2 and 4 post-infection, and on infected and controls on day 13. Temperature and weight were evaluated and blood samples were drawn on days 0, 1, 2, 3, 4, 7, 9, 11, and 13. Blood and tissues were processed by PCR to detect the gltA gene, and end titers of anti-*R. amblyommii* IgG were determined by indirect immunofluorescence. To evaluate protective immunity, another 5 guinea pigs were infected with *R. amblyommii*; after 4 weeks, these 5 infected guinea pigs (IGP) and 3 control guinea pigs (CGP) that had not been infected previously were inoculated with pathogenic *R. rickettsii*. Titers of anti-*Rickettsia* IgG and clinical signs were evaluated. After infection with *R. amblyommii*, IgG titers reached 1:512 at day 13 post-infection. *Rickettsia amblyommii* was detected by PCR in testicles on day 2. Some guinea pigs showed orchitis without other signs of disease. In the protective immunity assay, anti-*Rickettsia* IgG end titers after *R. rickettsii* infection were lower in IGP than in CGP. *Rickettsia rickettsii* was detected by PCR in testicles of CGP only. IGP did not exhibit disease or had only transient fever, while CGP showed severe disease and two died. Results demonstrate that *R. amblyommii* from Costa Rica produced infection, antibody response, and signs of mild pathology in guinea pigs after an experimental infection. Although more studies are required, *R. amblyommii* showed pathogenic potential and should not yet be excluded as a possible cause of disease. Also, its capacity to generate protective immunity may modulate the epidemiology and severity of *R. rickettsii* infections in areas where both species coexist.

722

ACE-DEX ENCAPSULATION OF ANTIGENS AND ADJUVANTS IS A POTENT DELIVERY PLATFORM FOR A *BURKHOLDERIA PSEUDOMALLEI* VACCINE

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Decades of modern research have failed to produce an effective vaccine against *Burkholderia pseudomallei*. There are several obstacles to a vaccine including lack of an appropriate target, lack of sufficient understanding of the immune response to *B. pseudomallei*, and lack of a vehicle capable of priming the appropriate arms of the immune system once the target is identified. Ace-DEX microparticles are a novel polymer carrier which is sensitive to acidic conditions; the microparticles are relatively stable at pH 7.4, but rapidly degrade after phagocytosis by antigen presenting cells. This acid sensitivity has been shown to yield enhanced CD8+ and CD4+ presentation of subunit antigen compared to other biomaterials. Ace-DEX encapsulation provides additional utility by allowing encapsulation of potentially toxic substances (if delivered freely) to be targeted to the site of action. Imidazoquinolines (e.g., resiquimod), for example are synthetic, FDA approved, immunostimulants that are agonists for TLR 7 and TLR 8, which are expressed within endosomal compartments of macrophages and multiple subsets of dendritic cells. Here we demonstrate that Ace-DEX encapsulation of antigens and adjuvants provide a potent delivery vehicle, producing a rapid and robust immune response inducing humoral and cell-mediated immunity. Mice were vaccinated with Ace-DEX particles encapsulating *B. pseudomallei* whole cell lysate and resiquimod on day 0 and 7 via sub-Q injection. On day 14 mice were challenged i.p. and followed for 26 days. Two groups had >90% survival up to day 13, and by the end of our study, two mice in each Ace-DEX-vaccinated group were sterile in all organs examined (blood, liver and spleen). All control mice succumbed to disease within 36 hours. Ace-DEX microparticles may represent a critical component to an effective *B. pseudomallei* vaccine.

723

RESEARCH OF IMMUNOGENICITY OF EXPERIMENTAL SERIES OF POLYVALENT VACCINE AGAINST LEPTOSPIROSIS IN BOVINE USING LABORATORY ANIMALS

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The purpose of our work was to research immunogenicity of new polyvalent vaccine against cattle leptospirosis using laboratory animals - rabbits. For this research, we used an experimental inactivated and concentrated 6-valent vaccine against cattle leptospirosis produced in leptospirosis laboratory of the Institute for Veterinary Medicine, NAAS. Antibody titers' formation dynamics was studied in microagglutination test (MAG) in serum laboratory animals (rabbits) serum on the 25th day after vaccination. MAG was carried out using 6 strains of *Leptospira* included into the vaccine composition: Sejroe (serovars polonica and hardjo), Hebdomadis, Icterohaemorrhagiae, Grippotyphosa, and Tarassovi. Serum dilutions from 1:50 to 1:1600 were tested in duplicate. The dilution where > 50% of the *Leptospira* were agglutinated was considered as the antigen titer. "The vaccine was deemed active if the antibody titer to *Leptospira* was not less than 1:100 in the blood sera of at least four of five vaccinated rabbits, each weighing 3.0 - 3.5 kg. It has been established that specific *Leptospira* antibodies (Sejroe (hardjo) 1:320±40; Grippotyphosa 1:640±80; Tarassovi 1:1040±187; Sejroe (polonica) 1:1120±161; Hebdomadis 1:1280±161 and Icterohaemorrhagiae 1:800±0) formed in all test animals after 25 days since intramuscular injections of the vaccine dose of 0.75 ml. Titers of antibodies to all strains of *Leptospira* included into the vaccine composition in the blood serum of vaccinated rabbits significantly exceeded a critical value of 1:100. It is an evidence of high immunogenic activity of the vaccine, which was reached through the improvement of an existing manufacturing technology and application of advanced methods of standardization of leptospirosis antigens in the polyvalent vaccine composition.

724

CHARACTERISTICS OF TRAVELERS TO ASIA REQUIRING COMPLEX MULTI-DOSE VACCINE SCHEDULES: RABIES AND JAPANESE ENCEPHALITIS PREVENTION

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Rabies and Japanese encephalitis (JE) pose risks to travelers to Asia, particularly those with longer and rural stays, outdoor activities, and visiting friends and relatives (VFR). Vaccines against both diseases require multiple doses and ≥21 days (rabies) or ≥28 days (JE) for completion. To describe interventions among travelers visiting affected areas in East (E), Southeast (SE), and South (S) Asia, we collected demographic and trip information for all patients seen during pre-travel consultations in BATMN clinics 03/08-07/10. Previous vaccination, travel reason, trip duration, and advice/immunizations were evaluated for travelers to E, SE, and S Asia. We calculated frequencies for categorical, and median and range for continuous variables. Of 15,317 patients, 5,091 (33%) traveled to E, SE, and S Asia. Of these 5,091, 52% were female and mean age was 36 years. Median trip duration was 17.8 days; 28% were traveling for >4 weeks. For rabies, only 5% had previously completed the series and 6% received complete series in the clinic. For JE, only 3% had previously completed the series, and 8% were immunized in clinic. Those traveling for education/research/missionary/ volunteer work had the highest proportion of rabies (17%) and JE (24%) vaccination; VFR travelers had the lowest (2%, 5%). Of 991 rabies vaccine-naïve travelers with trips >4 weeks, 583 (59%) were seen ≥21 days before travel. Similarly, of 994 JE vaccine-naïve travelers with trips >4 weeks, 461 (46%) were seen ≥28 days before travel. Most travelers received advice on vector precautions (97%); fewer were advised about rabies/animal contact (77-88%). In conclusion, frequency of rabies and JE vaccination was low among BATMN travelers, particularly for VFRs. Insufficient time to complete the series may have led to non-vaccination; travelers should schedule pre-travel consultations at least 4-6 weeks before travel. Cost may have also influenced vaccination decision. Health care providers should ensure rabies and animal bite prevention education in pre-travel consultations, in addition to emphasis on vector avoidance.

725

DRUG REPURPOSING INITIATIVE FOR NEGLECTED TROPICAL DISEASES

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Repurposing of approved drugs for neglected tropical diseases (NTDs) has proven to be a critical strategy to serve unmet medical needs. Approved drugs are well characterized, do not require expensive development programs needed for new drugs and are frequently active against diseases not explored by their original drug developer/sponsor. The landscape of repurposed drugs for neglected tropical diseases will be reviewed, and promising new candidates will be discussed. FDA is developing a web-based program to both capture and share the experience of the medical community using already approved drugs repurposed to treat tropical diseases. Users will be able to submit and query individual cases or clinical studies on drugs repurposed to treat neglected tropical diseases. In addition to the published literature, this program can help consolidate global experience on drugs that are either effective or not effective against NTDs. Safety experience using repurposed drugs to treat NTDs will also be reported to this database. Drugs with promising performance may be adopted by sponsors for more formal drug development. This approach is likely to enrich the armamentarium of drugs for those neglected diseases which have little commercial appeal to traditional drug developers.

726

SYMPTOMS AND CYTOKINE RESPONSES IN MALARIA MONO-INFECTION, DENGUE MONO-INFECTION AND MALARIA/DENGUE CO-INFECTION

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Over half of the world's population lives in an area endemic for malaria, dengue, or both. Despite overlapping endemicity, there is a lack of knowledge of the clinical presentation and host response associated with malaria/dengue co-infection. To identify symptoms and cytokine responses associated with co-infection, we conducted a retrospective matched comparative study within a febrile surveillance study conducted from 2002-2011 in the Peruvian Amazon Basin. For each co-infected subject (MAL-DEN), we selected 3 dengue mono-infected subjects (DENs) and 2 to 3 malaria mono-infected subjects (MALs) matched by location, time period (±3 months), gender, and dengue virus (DENV) serotype. Dengue was assessed using PCR and/or culture. Malaria was assessed using PCR and/or microscopy. We evaluated 20 symptoms using a standardized questionnaire. Seventeen inflammatory and regulatory serum cytokines and chemokines were quantified using Luminex xMAP technology. Matched comparison among MAL-DENs, MALs, and DENs was performed using conditional logistic regression for symptoms and random effects regressions for cytokine responses. Seventeen MAL-DENs (*Plasmodium vivax* in 14, *P. falciparum* in 3; DENV-1 in 2; DENV-3 in 15) were identified. Three symptoms were significantly different ($p<0.05$) when comparing the groups: 1) more abdominal pain in MALs vs DENs, 2) more myalgia in DENs vs MALs, and 3) more cough in MALs vs DENs. MALs had higher serum concentrations of interleukin (IL)-1β, IL-4, IL-6, IL-10, IL-12, IL-13, IL-17, granulocyte colony-stimulating factor, and γ-interferon ($p<0.05$) than either MAL-DENs or DENs. These differences remained after adjusting for age and days since symptom onset. In summary, MAL-DENs possessed a cytokine profile different from MALs but similar to DENs and did not have a higher frequency of symptoms compared to those with mono-infection. This suggests that many MAL-DENs may have had an acute DENV infection while also having asymptomatic chronic malaria, thereby creating a cytokine and symptom milieu similar to infection with DENV alone.

727

HUMAN IMMUNE RESPONSES AFTER CONTROLLED EXPOSURES TO SAND FLIES

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An innovative approach to *Leishmania* vaccine antigen discovery is exploration of the sand fly vector salivary molecules. In animal models, an effective cellular immune response to uninfected vector saliva has resulted in amelioration/protection from clinical leishmaniasis after parasite challenge. We hypothesize that humans develop immune responses to vector saliva deposited in the skin with sand fly (SF) bites. Our objective was to evaluate the clinical and immunologic responses in healthy human volunteers after controlled exposures to uninfected *Phlebotomus duboscqi* (Pd), a vector of cutaneous leishmaniasis, and *Lutzomyia longipalpis* (Ll), a vector of visceral leishmaniasis. Subjects were healthy 18-50 year olds with normal serum IgE levels, seronegative to SF saliva, and without a travel history to endemic SF regions. Fifteen subjects received bites from colony-raised Ll, 14 from Pd, with up to nine sessions/year. A variety of bite site reactions were noted including wheal and flare, vesicles and papules, delayed large local reactions at 48 hours, chronic pruritic papules lasting>2 weeks, and reactivations of prior feeding sites with subsequent SF bite exposures. Four subjects were removed from the study for bite-related adverse events. Low levels of serum IgG antibodies to SF salivary sonicate developed in most subjects following multiple exposures. SF saliva stimulated PBMC produced IFNγ and IL10. Skin biopsies of SF feeding sites showed varied histology, most commonly mononuclear perivascular and perieccrine infiltrates with occasional eosinophils, correlating with the clinical severity of the skin reactions. SF bites resulted in both reactogenic and immunogenic responses with variability in both the clinical reactions to SF bites and the systemic immune response. Immediate and delayed allergic reactions occurred. We are currently pursuing *Leishmania* vaccine antigen discovery to identify and avoid the reactogenic components of SF saliva, while concentrating work on the immunogenic salivary molecules.

728

AN ANALYSIS OF TRAVELERS TO ASIA SEEN IN THE BOSTON AREA TRAVEL MEDICINE NETWORK (BATMN)

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Asia is a popular travel region consisting of 5 sub-regions based on UN definition. We described travelers to Asia, compared them with travelers to other regions, evaluated differences in characteristics and interventions in the 5 sub-regions: South (S), Southeast (SE), East (E), Western (W) and Central (C) Asia. Demographic and trip information were collected for all travelers seen in BATMN clinics 03/08-07/10. We calculated frequencies and proportions for categorical variables and median and range for continuous variables. Travelers to Asia compromised 36% of travelers (5591/15307), with S Asia (35%) the most popular region and W and C Asia less visited (10%, 1%). The average age was 39y; travelers to W and C Asia were older (44y, 50y). Median trip duration was 17d (IQR 14-30); 28% planned trips >1mo and median time to departure was 23d (IQR 11-40). C Asia travelers had longer trips (26d; IQR 17-45); 49% traveled for >1mo and longer duration to departure (37d; IQR 15-52). Travelers were predominantly white (70%) or Asian (17%); a higher proportion to C Asia were white (90%). Travelers stayed in hotels (39%), hostels (31%) and local residences (28%). Tourism was the main reason for travel (46%), followed by VFR (18%) and business (18%). A higher percentage traveled for VFR (22%) and business (25%) to S Asia than other sub-regions. In C Asia, business (20%) and missionary/volunteer (14%) were also popular reasons for travel. Compared to travelers to other regions, Asia travelers were more likely to be male, older, born in Asia, more likely to travel for business and less likely for missionary/volunteer and have longer trip duration. Fewer received advice on swimming hazards, STIs and seat belts (83%, 68%, 87%) than other precaution topics. Azithromycin was prescribed more for self-treatment of diarrhea in travelers to S, SE and E Asia (79%, 65%, 48%) and ciprofloxacin to those visiting W and C Asia (63%, 77%). Atovaquone-proguanil was the most common anti-malarial prescribed for all regions. Typhoid immunization rates were higher in S Asia and SE Asia (77%, 75%), compared with E, W and C Asia (67%, 67%, 64%). JE and rabies vaccination rates were 6% and 8%. S Asia is the most popular of 5 Asian sub-regions to be visited by BATMN travelers. C and W Asia were less common destinations, their older age and longer trip duration may suggest different health risks such as road injuries, STIs. Pre-travel advice on swimming hazards, STIs, and seat belts need improvement.

729

RECOMBINANT *LEPTOSPIRA*L IMMUNOGLOBULIN-LIKE A PROTEIN-BASED IGM ELISA ASSAY FOR THE DIAGNOSIS OF LEPTOSPIROSIS

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Diagnosis of leptospirosis performed by culture or microscopic agglutination test (MAT) is laborious and not practical in clinical settings. The objective of the current study was to evaluate the clinical implication of a new immunoglobulin M enzyme-linked immunosorbent assay (IgM ELISA) using recombinant leptospiral immunoglobulin-like A (LigA) protein in endemic areas. A prospective study was conducted in a national infectious disease hospital in Metro Manila, the Philippines. Plasma and urine samples were collected from patients with clinically diagnosed leptospirosis from November 2011 through October 2012; all samples were tested by MAT, loop mediated isothermal amplification (LAMP) for *Leptospira*l rrs, and LigA-based and Patoc-based IgM ELISAs. The case was defined based on laboratory findings; 1) specific antibodies were detected with a single titer of >=400 or at least a 4-fold increase in the MAT titer between paired samples, or 2) LAMP was positive at least in 1 plasma and/or urine sample. The ELISA optical density (OD) values were compared between laboratory confirmed cases and 100 blood donor samples, and receiver operating characteristic (ROC) analyses were performed. A total of 72 clinically diagnosed cases were enrolled in this study, and paired samples were available for 36 cases. The median duration from onset to the collection of the first sample (acute phase) and the second sample (convalescent phase) were 7 (range, 3 to 40) days and 11 (6 to 46) days, respectively. Among all cases, 39 were laboratory confirmed; 38 (97.4%) were male, and median age was 30 (19 to 64) years. 29 had water-contact history, and 1 died. The area under ROC curve of LigA-based and Patoc-based IgM-ELISA were 0.82 and 0.7, respectively ($p<0.05$). When the mean+3SD OD value of 0.23 was used as the cut-

off limit, the sensitivity and specificity of LigA-based IgM-ELISA were 92.9% and 88.3%, respectively in acute phase and 92.3% and 99%, respectively in convalescent phase. Among the laboratory confirmed cases, LigA-based IgM-ELISA OD value reached the peak at 11 days after the disease onset and then declined. In acute phase, presence of jaundice, absence of diarrhea, lower hemoglobin, and higher creatinine were significantly associated with LigA positive cases but not with LigA negative MAT positive cases. LigA based-IgM ELISA is a useful diagnostic tool for leptospirosis in clinical settings in endemic areas.

730

EXPERIENCES WITH IMPLEMENTATION OF MALARIA RAPID DIAGNOSTIC TESTING AT PRIMARY HEALTH CARE LEVEL IN NIGERIA: IMPLICATIONS FOR SCALE-UP

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Nigeria commenced a phased programmatic deployment of rapid diagnostic tests (RDT) at the Primary Health Care (PHC) facility levels. However, against the backdrop of unexpected reactions to health intervention programmes like the polio vaccination campaigns, the acceptance, compliance and appropriate use of RDTs could not be assumed due to complex socio-cultural characteristics of the country. This implementation research was conducted to identify uptake, compliance and behavioural issues related to RDT use at the PHC level in Nigeria with their implications for scale-up of RDT deployment. A cross-sectional survey was conducted in 120 randomly selected PHCs across six states, across the six geopolitical zones of Nigeria in January 2013. Data on fever prevalence, malaria prevalence by RDT and adherence to test results were extracted from the medical records using a checklist covering Jun - Nov 2012. Compliance was the proportion of ACTs correctly prescribed in relation to RDT result. Health facility staff interviews were conducted to assess health workers (HW) prescription practices, knowledge and determinants of RDT use. A total of 1207 consecutive patient exit interviews to assess patient/caregiver's opinion of RDT use were conducted. There were 248,485 clinic attendees of which 129,272 (52.7%) presented with fever, but only 52,343 (39.8%) of these were tested using RDT kits with 31, 836 (60.8%) being positive for malaria. Of these30,674 (96.1%) received ACT while 8,418 (41%) of 20,507 RDT negative cases received ACT. Compliance rate was 81.7%. Of 118 HW's responses, about one-third each reported they would prescribe ACT (32.9%) or give antibiotics (29.8%) or refer (30.7%) when RDT is negative. Age of patients less than 5years (p=0.04) and "high" educational status of care givers (p=0.0006) were added determinants of health workers prescription of ACT to RDT negative patients. Overall, HWs had good knowledge of RDT. However in Enugu state, RDT was not being used because of a pervasive notion that it is not accurate. Among respondents who had RDT done, over 95% knew that RDT tested malaria, felt it was necessary and liked the test. The study demonstrated that RDT implementation at PHC level in Nigeria is feasible, safe, and acceptable with a significant compliance rate to RDT results. However pockets of challenges need to be addressed in the scale-up phase.

731

ETIOLOGY OF ACUTE FEBRILE ILLNESS IN FOUR HOSPITALS IN HAITI, APRIL 2012 - JANUARY 2013

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Acute febrile illness (AFI) causes significant morbidity and mortality worldwide. In Haiti, little is known about the etiologies of AFIs; the national surveillance system relies primarily on syndromic diagnoses, and clinicians often treat AFI patients empirically. In April 2012, we established laboratory-based surveillance for AFI at four hospitals -- two in Port Au Prince, one in Artibonite and one in Southeast -- to determine the major etiologies of AFIs and to monitor seasonal trends of AFIs. We defined AFI as a documented temperature ≥37.5ºC in a hospitalized patient at or within 24 hours of admission. Trained nurses collected blood specimens from and administered questionnaires to up to 10 AFI patients a week at each hospital. At the hospital, specimens were tested by rapid diagnostic tests for dengue and malaria. Blood was centrifuged, and sera were sent to the national laboratory to be tested for leptospirosis by immunodot and typhoid fever by TUBEX TF. From April 3, 2012 through January 16, 2013, we enrolled 1,284 AFI patients. The median age was 24 (Range: 1 month-99 years), 441 (36.1%) were <5 years old, and 630 (49.2%) were male. The largest number of patients (393; 30.6 %) was from La Paix Hospital (HUP) in Port-au-Prince. Overall, 131/1015 (12.9%) samples tested positive by TUBEX TF; 32/1079 (2.9%) were positive for malaria (23 [71.8%] from HUP); and 34/885 (3.8%) were positive for dengue (18 [52.9%] from HUP). Only 15/862 (1.7%) AFI cases were positive for leptospirosis. The median age (range) of patients with typhoid, malaria, dengue and Leptospirosis was 27 (3 months-78 years); 16 (2 months-65 years); 26 (1 month-65 years); and 28 (6-78 years), respectively. There was no marked variability in seasonal activity among the four pathogens. In a 10-month period in four hospitals in Haiti, typhoid fever, malaria, dengue and leptospirosis all contributed to hospitalized AFIs, but collectively were associated with less than 20% of all AFI patients. Future surveillance should expand testing to investigate other causes of AFI.

732

EFFECTS OF AN EDUCATIONAL INTERVENTION ON THE QUALITY OF THE INFORMED CONSENT OF PARTICIPANTS IN A CLINICAL TRIAL OF INTESTINAL HELMINTHS

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To obtain an informed consent is a crucial process for the ethical quality of clinical trials. However, participants of such researches generally have low comprehension regarding the information disclosed during the process of the informed consent. Also, they are under influences that interfere in their decision to participate in these researches, especially when such are carried out in developing countries. Therefore, it becomes necessary to create strategies to assure that the informed consent it will be a valid one, such as the accomplishment of educational interventions. Nevertheless, results from such interventions are controversial in affirming their impact in raising the informed consent quality, signalizing that further reflections, concerning different methods utilization, are needed. This study aim to analyze the impact of an educational intervention, based on a board game, on the quality of the informed consent from participants of an intestinal helminthiasis clinical trial that took part in the northeast region of Minas Gerais state, Brazil. The clinical trial goal was to evaluate the tolerability of adults residing in a helminthic endemic area to a functional

food with anti-helminthic properties. The study included 148 participants who were divided in two groups: experimental and control. On the first group, the subjects have joined on a board game before signing the informed consent form, while the second group just signed the document. In order to evaluate the informed consent quality, a structured questionnaire, that measures participant's knowledge concerning information of the informed consent and the presence of influences in the decision making process, was applied. Comparisons between the groups were done by Chi-Square and Mann-Whitnney test (significance level of 5%). Experimental group participants have showed larger knowledge regarding information of the informed consent and have presented lower influence over their decision to participate in the clinical trial. We have concluded that an educational intervention, that has applied a board game as an instrument, was able to improve the quality of the informed consent of participants in a clinical trial. It is also concluded that educational interventions should be associated to the process of informed consent achievement in order to favor a truthfully informed consent to participants of clinical trials.

733

TRAVELERS' DIARRHEA RISK FACTORS AND INCIDENCE OF POST-INFECTIOUS IRRITABLE BOWEL SYNDROME (PI-IBS) IN A LARGE PROSPECTIVE COHORT OF DEPARTMENT OF DEFENSE BENEFICIARIES (TRAVMIL)

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There is limited prospective data regarding risk factors for and outcomes related to traveler's diarrhea (TD). Between 1/2010 and 11/2012, Department of Defense beneficiaries traveling outside the US for < 6.5 months were invited to participate in a prospective, multicenter cohort study - TravMil. Participants received a pre- and post- travel survey, and could opt into an illness diary completed during travel and a follow-up survey at 3, 6, 9 and 12 months post-travel. Standard definitions were used to assess for TD, and diagnosis of irritable bowel syndrome (IBS) was based on the modified Rome III criteria. Of the 838 participants who returned the questionnaires, 198 (24%) met criteria for TD: 9 (1.1%) had dysentery, 48 (5.7%) had acute febrile watery diarrhea and 141 (16.8%) had acute watery diarrhea. The highest incidence density rate (IDR) was found in Africa (7 cases per 100 person-weeks of travel (95% CI 5.4-9.4 cases per 100 person-weeks of travel)) and South Asia (6.1 cases per 100 person-weeks of travel (95% CI 3.6-9.8 cases per 100 person-weeks of travel)). Significant risk factors associated with TD in the multivariate model included: trip duration ≤ 2 weeks (IDR ratio 2.7 (95% CI: 1.9-3.8; p<0.01), leisure travel (IDR ratio 1.5 (95% CI: 1.1-2.1; p=0.02) and ingesting food prepared by street vendors or at home by local population (IDR ratio 1.4 (95% CI: 1.02-1.94; p=0.035). Among patients who did not have symptoms of IBS at baseline (n=803) and completed at least one follow-up survey (n=481), TD was not associated with an increased incidence of post infectious IBS at 12 months (IBS in patients with TD: 6.5% (7/108); IBS in patients without TD: 4% (15/373); relative risk 1.6 (95% CI: 0.67-3.85). Travelers' diarrhea remains a frequent occurrence among travelers to Africa and South Asia. The incidence of PI-IBS post-travel was similar to rates reported in prior studies. We were unable to demonstrate an association between TD and post-infectious IBS due to a small sample size.

734

AN EPIDEMIOLOGICAL SURVEY OF PREGNANT WOMEN IN THE CHAGAS ENDEMIC REGION OF THE BOLIVIAN CHACO: A PRELIMINARY REPORT

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Chagas disease is a major health problem throughout Central and South America and is endemic across 60% of Bolivia. An estimated 26% of new cases are considered to be from congenital transmission. Current diagnostics and national screening protocols are estimated to miss 50% of congenital cases. Here we present the epidemiological data collected from a study of pregnant women admitted to Hospital Municipal Camiri in Camiri, Bolivia evaluating the prevalence of and risk factors for *Trypanosoma cruzi* infection. The women were recruited prior to giving birth and asked about their health history, socio-economic status, and risk factors for infection with *T. cruzi.* The mothers' venous blood was screened by the InBios Trypanosoma Detect Rapid Test. Mothers were considered to be *T. cruzi* infected if positive by Indirect Hemaglutination Assay and ELISA. To date, 174 women (mean age ±SD, 24.8 ±6.9) have entered the study of whom 79 have *T. cruzi* infection (45.4%). A significant association was shown between increasing age and *T. cruzi* infection in the mothers (p=0.0007). Overall, 37.6% of the women live in rural areas, and 27.9% reported living in a house infested with the triatomine vector. Women from rural areas were more likely to be infected with *T. cruzi* (p=0.038), and reported greater triatomine presence in their house (69.4% in rural vs 2.91% in urban areas). Women who lived more than 10 years in an infested house had a significantly higher rate of *T. cruzi* infection compared those who lived fewer than 10 years in an infested house (Odds ratio [OR] 4.45; 95% Confidence Interval [CI]: 2.21-9.02). Women who reported having a family member with Chagas or having a history of a triatomine bite were more likely to be *T. cruzi* infected (p=0.001, p<0.0001). Preliminary analyses suggest women of childbearing age living in rural areas near Camiri have a high prevalence of *T. cruzi* infection. Forthcoming data on congenital transmission rates in our unique and highly seropositive study group will allow multivariate analyses of risk factors for congenital transmission.

735

THE POTENTIAL IMPACT OF IMPROVING APPROPRIATE TREATMENT FOR FEVER ON MALARIA AND NON-MALARIAL FEBRILE ILLNESS (NMFI) CASE MANAGEMENT IN UNDER-5S: A DECISION-TREE MODELLING APPROACH

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As international funding for malaria programmes plateaus, limited resources must be rationally managed. Although previously advocated, a policy of presumptive malaria treatment has led to widespread unnecessary prescription of antimalarials to non-malarial febrile illness (NMFI) cases. Hence it is critical to better understand how to implement interventions such as first-line Artemisinin Combination Therapies (ACTs) most effectively through an existing system, ensuring those who need treatment receive it, and that those who do not, are not needlessly treated. We developed a decision-tree tool to estimate the impact of improving health systems factors on rates of appropriate treatment for fever and on use of ACTs,

and to also evaluate the impact in Tanzania of the revised WHO malaria 2010 guidelines advocating diagnostic-led management. At baseline, 49% malaria cases attending a clinic receive ACTs (95% Uncertainty Interval: 40.6-59.2%) however 44% (95% UI: 35-54.8%) NMFI cases also receive ACTs. Increased treatment-seeking was the most effective step in increasing the proportion of all febrile cases correctly managed, but had no effect in improving case management of those patients attending the clinic. Provision of 100% ACT stock led to a 28.9% increase in malaria cases treated with ACT, but also increased overtreatment of NMFI, with 70% NMFI cases (95% UI: 56.4-79.2%) receiving ACTs and so an overall 13% reduction (95% UI:5-21.6%) in correct management of febrile cases. Increased availability or use of diagnostics had little effect on malaria management, but did significantly reduce NMFI overtreatment. Modelling the impact in Tanzania of the revised WHO guidelines, NMFI overtreatment decreased by 35% (95% UI: 31.2-39.8%), but malaria cases receiving ACTs reduced by19.5% (95% UI: 11-27.2%), due to a fourfold decrease in cases that were untested or tested false-negative (42.5% vs. 8.9%) and hence untreated. Multi-pronged intervention strategies were most effective to improve malaria treatment without increasing NMFI overtreatment. As malaria transmission declines, health system interventions must be guided by whether the management priority is an increase in malaria cases receiving ACTs (reducing the treatment gap), reducing ACT waste through unnecessary treatment of NMFI or expanding appropriate treatment of all febrile illness.

736

ETIOLOGY OF INFECTIONS OF THE CENTRAL NERVOUS SYSTEM AMONG CHILDREN IN MBARARA, UGANDA

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Knowledge of etiological agents causing central nervous system (CNS) infections among children is essential to the development of guidelines for case management. This is especially true where there is little investigative capacity. We conducted a prospective descriptive study of the etiology of CNS infections in children two months to 12 years of age admitted to Mbarara Regional Referral Hospital with fever or a history of fever and at least one sign of CNS involvement. Clinical examination and biological sampling were performed upon admission. Pathogens were identified from CSF and blood samples following microbiological analysis, molecular diagnosis and serology. We diagnosed malaria using rapid diagnostic test and blood smears. Children were clinically assessed at discharge and then followed for any neurological sequellae.Between August 2009 and October 2012 we recruited 480 children with clinically suspected infection of the CNS, with a median age of 2 years (range 1month-5 years). The most important clinical symptoms at admission were prostration (78.5%), reduced consciousness (71.0%) and seizures (50.8%). Eighty-eight children (18%) died in the hospital. Malaria accounted for 166 (35%) of the laboratory confirmed diagnoses with a Case Fatality Ratio (CFR) of 13% while 49 (10%) of the children had culture or PCR-proven bacterial meningitis with a CFR of 16%. The most common pathogens were *Streptococcus pneumoniae* (33) followed by *Haemophilus influenzae* (7) and *Salmonella spp.* (6). Preliminary results of viral PCR and serology revealed 4 HHV6 and 1 mumps infections. No etiological agent could be identified in 198 patients (41%). Among those who survived, the proportions of neurological sequellae were 23% at discharge, 17% at one month and 11% at six months. Malaria is the main cause of CNS infections among children in Mbarara. These infections remain an important cause of mortality and morbidity and a treatment challenge due to diagnostic difficulties.

737

INTRODUCTION OF ZINC WITH ORS FOR THE TREATMENT OF PEDIATRIC DIARRHEAS: THE GHANA CASE STUDY

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Key lessons learned through the public-private partnership in Ghana for increasing the use of ORS and zinc for the treatment of pediatric diarrheas will be described. Diarrheal disease is highly prevalent among children under five in Ghana, at a rate of about 20 percent, and accounts for an estimated 9 percent of childhood deaths. In 2011 the Government of Ghana adopted WHO/UNICEF guidelines recommending treatment of pediatric diarrhea with 10 days of zinc plus oral rehydration solution (ORS). The Ghana Health Service with USAID partners, USAID Strengthening Health Outcomes through the Private Sector (SHOPS) Project and Johns Hopkins Center for Communication Programs, used a collaborative approach to maximize availability of, demand for, and use of ORS plus zinc. Ingredients for success included: Public sector leadership to establish a conducive policy and regulatory environment; stakeholder engagement from both public and private sectors to develop training materials for health professionals and conduct joint training of trainers; training of private sector providers in diarrhea management including licensed chemical sellers, pharmacists and clinicians; grants to local pharmaceutical manufacturers to increase availability of quality, affordable pediatric zinc and marketing/detailing to private retailers and providers; a strategic and innovative mass media campaign increased awareness, demand and use; and grants to local NGOs to promote use of ORS plus zinc through interpersonal contact with caregivers and community mobilization activities. In 2012 mystery client survey and in-depth interviews, over 80% of providers mentioned zinc as their preferred diarrhea treatment and 65% actually prescribed zinc with ORS as treatment. Private sector sales of zinc tablets increased ten-fold after training providers and an additional 300% after the media campaign, treating over 1 million children with diarrhea during 2012. In a single year, significant progress in scaling up diarrhea treatment can be realized through targeted partnerships with the private sector.

738

CLINICAL SIGNS AND SYMPTOMS ASSOCIATED WITH CHOLERA PATIENTS IN HAITI, 2012

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As of March 24, 2013, over 651,339 patients had been reported to the national cholera surveillance system in Haiti since the outbreak began in October, 2010. All patients with acute watery diarrhea who present to cholera treatment facilities (CTFs) are treated as presumptive cholera patients. We used data from a sentinel surveillance system for diarrhea in Haiti to identify signs and symptoms associated with cholera. We conducted surveillance for diarrheal illness in four hospitals in Haiti located in three departments: two in Ouest, one in Artibonite and one in Sud-Est. In each hospital, nurses recruited 10 patients per week who had at least 3 episodes of acute watery diarrhea within 24 hours with onset in the prior 7 days. Patients who had taken antibiotics were excluded. For each patient, we collected demographic and clinical information and a stool sample, which was sent to the National Public Health Laboratory where it was cultured for *Vibrio cholerae*. From April 1, 2012 -- December 12, 2012, we enrolled 921 patients, of whom 490 (53.2%) were male. The mean age was 23 years old (Range: 1 month–99 years), 187 (20.3%) were <5 years old, and 574 (62.3%) had cholera confirmed by culture. Compared to cholera-negative patients, cholera patients were more likely

to be severely dehydrated (69.1% vs. 41.0%; OR=3.2; 95% CI=2.3-4.4), to have had >10 episodes of diarrhea in a 24-hour period (57.1% vs. 32.2%%; OR = 2.8; 95% CI=2.0-3.8), to have vomiting (85.7% vs. 67.8%; OR =2.8; 95% CI=2.0-3.9), and muscle pains (31.4% vs. 16.5; OR=2.3; 95% CI=1.6-3.3). Both vomiting and 10 or more stools in a 24-hour period were more strongly associated with cholera among patients <5 years old than among older patients (OR 4.7 vs. 1.5 for vomiting; and OR 3.3 vs. 1.7 for ≥10 stools per day, respectively). In settings where diagnostic testing is not immediately available, evaluating certain signs and symptoms in patients with acute watery diarrhea may help clinicians more accurately identify patients with cholera.

739

UNDERSTANDING DETERMINANTS OF INTERNATIONAL TRAVEL BEHAVIOR FOR PREVENTION OF IMPORTED DENGUE

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Travel medicine uses a risk assessment framework of social determinants of health for high-risk visiting friends and relatives (VFR) travelers to determine risk of travel-associated diseases. Such assessments are subject to bias and inaccuracy because these lack a social-ecological perspective of factors that influence travel behavior. Imported Dengue is a concern due to risk of secondary transmission and outbreaks in non-endemic regions. For U.S. travelers, Dengue prevention includes mosquito avoidance practices (MAP) and passive surveillance. Identifying determinants of MAP can improve Dengue prevention. A mixed-methods project identified and described determinants of MAP in U.S. VFR travelers. Next, a multi-case study of travel cohorts to Trinidad, Brazil and Thailand identified social/physical environmental influences on MAP in a cross-case content analysis. Finally, an interpretive phenomenology study described the meaning of going home for VFR travelers. Preceding pilot studies revealed determinants of intended MAP in a survey of travelers to Trinidad Carnival. Survey analyses included an exploratory factor analysis, Fisher's exact test, logistic regression and coding of open-ended questions. Factors associated with lack of intended MAP were carnival dedication (p=0.025) and feeling at home in the travel destination (p=0.007); VFR status was not significant (p=0.567). Semi-structured interviews revealed MAP are associated with risk perception, cultural familiarity and cultural embeddedness, irrespective of VFR status. Results included a *Cultural Embeddedness and MAP* model. Constructs include risk distractions, risk motivators, and objective and subjective decision-making for MAP. Multi-level prevention strategies would complement recommendations for MAP. Dengue prevention should include a Dengue Early Warning Surveillance System and fever-screening programs in high-risk cities and airports. Furthermore, VFR terminology does not accurately depict high-risk travelers, so adoption of the concept of '*Cultural Embeddedness*' should improve risk assessments of high-risk travelers.

740

ENHANCING HEALTH CARE WORKER CAPABILITIES TO DETECT AND CARE FOR PATIENTS WITH MONKEYPOX

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Monkeypox has been a nationally notifiable disease in the Democratic Republic of Congo (DRC) since 2000, with oversight for surveillance and response activities provided by the *National Program for Monkeypox and Hemorrhagic Fevers*. In October 2010, the CDC Poxvirus Team initiated collaboration with the Ministry of Health and the Kinshasa School of Public Health to identify methods to enhance monkeypox surveillance and containment capacity, focusing on Tshuapa District in Equateur Province. In early February 2011 and again one year later, five representative health care workers from each of the 12 health zones in Tshuapa District attended a monkeypox surveillance training program. These individuals were expected to then replicate training for subordinates in their respective health care settings. Participants received training on monkeypox recognition, specimen collection, infection control, and in surveillance methods. In conjunction with the training program, an evaluation of training effectiveness and a brief needs assessment were performed using a self-administered written survey tool. Fifty-eight healthcare workers participated in the pre and post-knowledge evaluations. After training, more people correctly associated lymphadenopathy and deep well-circumscribed lesions (vs. superficial lesions) with monkeypox than prior (22/57 vs. 53/57, respectively). An improvement was also observed in the correct identification of vesicular fluid and lesion crusts (rather than blood) as preferred samples for laboratory testing. The lack of sampling kits, case forms, and personal protective equipment (PPE) were commonly indicated by survey respondents as impediments to MPX case investigations. When specifically asked how often the necessary PPE was available for collection of MPX samples, 47.7% responded 'sometimes' and 3.1% responded 'never', suggesting that lack of PPE is a notable barrier to the effective performance of monkeypox surveillance activities. This evaluation highlights several key areas for improvement of MPX surveillance in Tshuapa.

741

SUSCEPTIBILITY TO, AND RISK FACTORS FOR, HEPATITIS E VIRUS INFECTION AMONG A REFUGEE POPULATION - SOUTH SUDAN, 2012

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Hepatitis E Virus (HEV) infection causes outbreaks in areas with poor water, sanitation and hygiene (WASH) conditions. Mortality rates range from 0.2% to 4.0%, but can reach 30% among pregnant women. In September 2012, an HEV outbreak was confirmed in Jamam refugee camp, South Sudan. At the time, there were approximately 15,000 refugees living in Jamam camp with 668 suspected/confirmed cases and

22 deaths. CDC was asked to assess the prevalence of infection and risk factors for HEV. A cross-sectional serosurvey was conducted in November 2012. Two individuals ≥3 years were randomly selected from a simple random sample of households. We collected serum for anti-HEV antibody testing and information on hygiene practices and exposures from each participant. We identified risk factors for HEV infection through a nested case-control analysis based on serology results; cases were IgM positive individuals while controls were unmatched individuals, negative for both IgM and IgG. A total of 443 individuals participated in the serosurvey; 21.7% had recent HEV infection (IgM positive), 24.1% had past infection (IgG positive), and 54.3% had no evidence of HEV infection (IgM and IgG negative). A total of 106 cases and 215 controls were included in the nested case-control study. Most cases were between 15-45 years and there was no significant difference between sexes. Individuals reporting close contact with a person with jaundice and those taking care of animals had 1.8 (95% CI: 1.1, 3.1) and 2.3 (95% CI: 1.03, 4.9) times the odds, respectively, of recent HEV infection compared to those unexposed. No other exposures were associated with infection. In conclusion, over 50% of Jamam refugees were susceptible to HEV infection four months after the outbreak started, highlighting the risk for continued transmission. Contact with a person with jaundice may play a role in HEV transmission in this population. The role of animal caretaking needs further investigation. Personal hygiene and WASH interventions should be scaled up in the face of a prolonged HEV outbreak.

742

ECOLOGICAL AND EPIDEMIOLOGICAL STUDY OF VIRAL AND *RICKETTSIA*L PATHOGEN PREVALENCE IN TICKS AND MOSQUITOES IN THE NORTHERN PART OF AZERBAIJAN

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This project, which began in October 2012, aims to: conduct ecological and epidemiological studies of ticks, sandflies, and mosquitoes that may act as disease vectors of viral and rickettsial pathogens northern Azerbaijan; identify by PCR the viruses and rickettsia carried by these vectors; map vector and pathogen foci using Geographic Information Systems (GIS); and develop strategies for vector and disease surveillance and control based on these results. Data collected by this project will be used to test the hypothesis that understanding the ecology of arthropod disease vectors - ticks, sandflies, and mosquitoes -can assist public health authorities in anticipating the occurrence of the diseases they harbor and may transmit to humans and animals. Research will be conducted in northern regions of Azerbaijan, including Khachmaz, Guba, and Gusar, to investigate the following pathogens: Crimean-Congo hemorrhagic fever virus; tick-borne encephalitis virus; West Nile virus; Toscana virus; Sindbis virus; and various *Rickettsia* species, including that which causes Q-fever. Expected outcomes of this study: determination of the distribution of various tick, sandfly, and mosquito species within selected areas, including host and environment dependent distribution; deployment of up-to-date GIS technology to enhance surveillance, epidemiological, and analytical capabilities; full employment of DTRA-renovated facilities in Azerbaijan, including those located outside of Baku; exercise of knowledge gained from previous DTRA-sponsored research projects; application of modern molecular analysis techniques and expansion of project personnel's capabilities in these methods; and creation of an archival reference collection of arboviral and rickettsial genetic material for future use in identification, research, and training efforts. Initial sample collection was conducted in October 2012, with the quantification and identification of vectors collected still to be completed. We anticipate having preliminary testing results available by the date of the conference.

743

EFFECTIVENESS OF LONG-LASTING PERMETHRIN IMPREGNATED UNIFORMS FOR TICK BITE PREVENTION AMONG FORESTRY, PARKS AND WILDLIFE WORKERS IN NORTH CAROLINA

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Outdoor workers, because of frequent exposure in tick habitats, are at high-risk for tick-borne diseases. Adherence to NIOSH recommended tick-bite prevention methods is poor. While self-applied permethrin treatment of clothing is highly protective against many arthropod vectors, the need for frequent reapplication lessens adherence. We evaluated the protective effectiveness of long-lasting permethrin impregnated (LLPI) uniforms among a cohort of North Carolina outdoor workers. A double-blind randomized controlled trial was conducted to determine the effectiveness of LLPI uniforms for the prevention of tick bites among outdoor workers from North Carolina State Divisions of Forestry, Parks and Recreation, and Wildlife. 159 subjects were randomized; uniforms of participants in the treatment group were factory-impregnated with long-lasting permethrin while control group uniforms received a sham treatment. Participants continued to engage in their usual tick-bite prevention activities and provided weekly tick bite logs during two tick seasons. Questionnaires were completed annually. Study subjects reported 1,045 work-related tick bites over 5,251 person-weeks of follow-up. The mean number of reported tick bites in the year prior to enrollment was similar for both the treatment and control groups, but markedly different during the study period. The effectiveness of LLPI uniforms for the prevention of work-related tick bites was 82.8% (95% Confidence Interval (CI): 67.7% to 90.8%) for the first year of follow-up and 38.4% (95%CI: -51.4% to 74.0%) for the second year of follow-up. These results indicate that LLPI uniforms are highly effective for at least one year in deterring tick bites in the context of existing tick bite prevention measure usage by outdoor workers.

744

PREVALENCE OF *RICKETTSIA, EHRLICHIA* AND *BORRELIA* IN ARTHROPODS IN GEORGIA

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Rickettsia, Borrelia and *Ehrlichia* are arthropod-borne pathogens widely distributed throughout the world. These pathogens cause diseases with similar clinical signs, they can share the same transmitting vector; co-infection is also possible that makes difficult to diagnose. Preliminary study showed a relatively high infection rate for spotted fever group *Rickettsiae* among ticks in Georgia. No studies had been conducted on *Borrelia* and *Ehrlichia* in vectors. The goal of the study was to evaluate the prevalence of *Rickettsia, Ehrlichia*, and *Borrelia* species in Georgia. 500 single species vector pools, representing 13 species collected from 3 regions of Georgia were studied using qPCR. Analysis of the results revealed that 47% of sample pools were positive on *Rickettsia*, 23% on *B. burgdorferi*; 19% of the pools were positive for both *Rickettsia spp.*, and *B. burgdoferi*. Distribution of *Rickettsia* was similar in all regions. The distribution of *B. burgdorferi* was not uniform. *Rickettsia* and *B. burgdorferi* were found in all tick species with the exception of ticks of the genus *Argasidae* (possibly due to small number of these vectors - 32 tick samples were tested). Of all tick species studied, minimum infection rates (MIRs) for both *Rickettsia* and *B. burgdorferi* were highest for *Hyalomma* genus. For *Rickettsia, H.*

plumbeum had the highest MIR (2.19%); the highest MIR (2.15%) for *B. burgdorferi* was observed in H. aegyptum. The flea pools that were inadvertently analyzed in this study were *Rickettsia* and *B. burgdorferi* negative, but this does not discount the possibility that fleas in Georgia carry these pathogens as the sample size for these vectors was extremely limited. This study is still ongoing and more samples from other regions of Georgia are under investigation. Screening ticks for these bacterial agents has provided insights regarding the distributions and endemicity of potentially pathogenic and emerging tick-borne agents. Continued study and monitoring will play an important role in public health assessment for related disease risks.

745

IDENTIFYING A REFERENCE GENE FOR *RICKETTSIAE* USING *RICKETTSIA BELLII* AS A MODEL

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Rickettsioses are caused by obligate intracellular bacteria transmitted by hematophagous arthropods such as ticks. In rare occasions, ticks have been documented to be superinfected with two spotted fever group and an ancestral group rickettsia, as reported previously. Tick-borne rickettsioses are an emerging global health concern. *Rickettsia spp.* formerly recognized as nonpathogenic maybe contributors to the emergence of rickettsioses. With the emergence of tick-borne rickettsioses, there is a need to understand *rickettsiae* and how they respond to their arthropod and human host and to the environment at a molecular, cellular, and immunological level. In this research, we focus on using *Rickettsia bellii* RML-369C, a non-pathogenic bacterium, as a model to understand the transcriptional control as it grows in tick and mammalian cell culture. *R. bellii* is safer to handle and grow in laboratory settings, and grows voraciously in ticks and mammalian cell culture similar to pathogenic *rickettsiae*. Our overall aim is to study the transcriptional response of *rickettsiae* when they first infect a tick or mammalian host in nature to understand how they respond during infection and establishment of their hosts. This research aims to identify stable reference genes to analyze transcriptional responses of *R. bellii* grown in ticks and mammalian cells as a laboratory replica of rickettsial infection in nature. We used a two-step quantitative reverse transcription PCR to screen 5 housekeeping genes: *atpB*, *gltA*, *gyrA*, *infB*, and *tlc5* for use as reference genes. Out of the 5 genes assessed using Normfinder, we identified *gyrA* as the most stablely expressed gene throughout a 72 hour post infection growth period in tick cell culture. A best combination of *gyrA* and *tlc5* was identified as being suitable for transcriptional analysis.

746

VIRAL AND *RICKETTSIA*L SCREENING OF IMMATURE TICKS COLLECTED IN MISSOURI

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The novel phlebovirus, *Heartland virus*, was identified in two patients from Missouri who had both been bitten by ticks 5 to 7 days before the onset of their symptoms. In September 2012 our laboratory group went on a collection trip to Missouri with the goal of isolating *Heartland virus* in ticks. We identified three geographically relevant collection sites. At the three locations 1267 larval ticks and 2 nymphal ticks were collected. The ticks were identified by morphology and then homogenized for pathogen screening. Of the collected ticks there were 93.9% *Dermacentor albipictus*, 5.8% *Amblyomma americanum*, and 0.3% *Ixodes scapularis*. Vero, VeroE6, and DH82 cells were infected with the homogenized ticks however no CPE was detected. Primers specific for *Heartland virus*, *Powassan virus*, and *Deer tick virus* were used in the viral PCR screening, but none of the samples from the collection sites were positive for virus. DNA extracts were screened for bacterial pathogens, and at one location a larval and a nymphal pool of ticks were both *Rickettsia*-positive. Sequence analysis demonstrated that there was 100% sequence identity between the two positive samples and the *Candidatus Rickettsia amblyommii ompA* and *citrate synthase* genes. Morphologic tick identifications were verified by mitochondrial 16S rRNA sequence analysis which confirmed that the *R. amblyommii*-positive samples were isolated from *A. americanum* ticks. Our data indicates the occurrence of transovarial transmission of *R. amblyommii*, as evident by positive larval ticks collected at a single location. To date no definitive role has been defined for *R. amblyommii* in human pathogenesis, but a recent study has shown that *A. americanum* ticks parasitizing humans are frequently infected with *R. amblyommii* (Jiang et al., 2010). Because other *Rickettsia* species, such as *R. parkeri*, were initially thought to be endosymbionts but were later shown to be pathogenic, it is important to continue evaluating the potential public health threat that *R. amblyommii*-infected *A. americanum* ticks pose to the humans they parasitize.

747

DETECTION OF A *RICKETTSIA* CLOSELY RELATED TO *R. MONACENSIS* IN *IXODES BOLIVIENSIS* FROM COSTA RICA

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Rickettsia monacensis was officially described in 2002 from *Ixodes ricinus* ticks in Germany. It has now been detected in most areas of Europe and has been associated with Mediterranean spotted fever-like human rickettsiosis in Spain and Italy. *I. pacificus* and *I. scapularis* from North America also contain similar *Rickettsia sp.*, which have not been fully described. We report the presence of a *Rickettsia sp.* in *I. boliviensis* from Costa Rica that is very closely related to *R. monacensis*. In February 2012, ticks were collected from domestic dogs in the province of Heredia, Costa Rica. Specimens of the same species and from the same dog were pooled, and DNA was extracted. Pools were analyzed by *Rickettsia spp.* specific PCRs that detect fragments of the gltA, htrA, ompA, and ompB genes. Amplicons from positive pools were sequenced, and BLAST searches and phylogenetic analyses were performed. *I. boliviensis* were collected from 6 of 10 dogs evaluated. DNA of *Rickettsia spp.* was detected in all 6 pools of *I. boliviensis*. At least 2 of the tick pools contained gltA fragments that were 100% homologous with each other, and BLAST analyses determined 99.7% (365/366) homology to *R. monacensis* IrR/Munich. BLAST and phylogenetic analyses of gltA, ompA, and htrA amplicons confirmed that the *Rickettsia sp.* IbR/CRC present in *I. boliviensis* ticks from Costa Rica groups closely with *R. monacensis* and *Rickettsia sp.* from *I. pacificus* and *I. scapularis*. This is the first detection of a *Rickettsia* in *I. boliviensis* ticks of Central America. Further analyses are required to determine if *Rickettsia sp.* IbR/CRC is a genotype of *R. monacensis*, a genotype of the *Rickettsia sp.* in *Ixodes* from North America, or a different species yet to be described. Considering that *I. boliviensis* can bite humans and that *R. monacensis* and other closely related species have been associated with human disease, it is important to characterize and determine the pathogenic potential of this *Rickettsia*.

748

MODELING DIFFERENTIAL INVASION TRAJECTORIES OF TICK-BORNE DISEASES ACROSS THE NORTHEASTERN U.S.

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The ongoing invasion of two tick-borne diseases, Lyme disease and human babesiosis, in the Northeastern United States presents a significant public health risk as well as a unique opportunity to study the dynamics of ongoing pathogen emergence. Although both pathogens share the

same tick vector and an overlapping community of vertebrate reservoir hosts, expansion of babesiosis has markedly lagged that of Lyme and is endemic in a much smaller range. Invasion of tick-borne pathogens is a product of local, small-mammal mediated spread and long-distance, bird-mediated spread. However, the relative contribution of different hosts to pathogen dispersal and the factors driving the differential expansion trajectories for the two pathogens remain unknown. We use a hierarchical Bayesian framework for testing mechanistic hypotheses that describe the spatio-temporal distribution of human cases of each disease across the Northeast. The model is parameterized with data compiled from state and county health departments from 1984-2011 for Lyme disease and from 1990-2011 for babesiosis. Three model structures are developed for comparison. Specifically, we examine the relative importance of local versus long-distance spread processes for predicting invasion dynamics of the two diseases. We also identify climate, landscape and vector biology factors significant in predicting pathogen diffusion. The best-fitting model structure included both spatially variable local diffusion and long-distance spread. Diffusion of babesiosis was found to be consistently slower than that of Lyme across the Northeastern emergence focus. This data-driven mechanistic framework is a simple but accurate approach to studying the invasion dynamics of pathogens maintained in complex ecological cycles such as the tick-borne infections presented here. Our method can be used to predict sites of probable invasion, thus identifying spatial targets for enhanced surveillance and control measures.

749

PHLEBOTOMUS ORIENTALIS SALIVARY ANTIGENS - IDENTIFICATION, CHARACTERIZATION AND EXPRESSION

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Sand flies (*Diptera: Phlebotominae*) are vectors of *Leishmania* (Trypanosomatidae), the causative agents of cutaneous and visceral leishmaniasis. During the blood feeding, sand fly females inject saliva into the host skin to overcome host haemostatic mechanism. Repeated exposures to sand fly saliva elicit anti-saliva antibodies that could be used in epidemiological studies as a marker of exposure to assess the risk of *Leishmania* transmission and the effectiveness of anti-vector campaigns. The anti-saliva immunity has been also shown to protect the host from *Leishmania* infection, thus salivary proteins are considered as candidates for transmission blocking vaccine. The main aim of this study was to characterize and express salivary gland antigens of *Phlebotomus orientalis*, the important vector of life-threatening visceral leishmaniasis in East Africa. The major antigens were determined by SDS-PAGE and immunoblot using antibodies from dogs and humans repeatedly bitten by this sand fly species.. Based on the cDNA library and mass spektrometry (MALDI TOF-TOF), eight antigens from five different protein families were identified. All of these proteins with molecular weight ranging from 26 kDa to 42 kDa are antigenic for dogs but only four (apyrase, yellow-related protein, antigen 5-related protein, D7-related protein) are antigenic for humans. These four antigens were expressed in the bacterial expression system. Recombinant products will be compared in their ability to bind specific antibodies in sera from hosts repeatedly bitten by *P. orientalis* to select candidate antigen(s) usefull for larger epidemiological studies.

750

EFFECTS OF FOREST PATCH SIZE ON ABUNDANCE OF DEER TICKS (*IXODES SCAPULARIS*) AND RECOGNITION OF RED-BACKED VOLES AS MAJOR RESERVOIRS OF *BORRELIA* AND *ANAPLASMA* IN GRAND FORKS COUNTY, NORTH DAKOTA USA

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Geographic range of the deer tick, *Ixodes scapularis*, has expanded in recent years. Deer ticks are forest ticks and historically considered not to occur as far west as North Dakota. However, a statewide survey conducted in 2010 found breeding populations of deer ticks in the Northeast region of the state. This region is under intense agricultural production but within the expanse of field crops there are islands of forested areas. In 2012 six forested areas in Grand Forks County, ranging in size from 7 to 349 hectares, were surveyed for deer ticks. Ticks were collected via dragging and from small mammals. There was a significant positive correlation between forested patch size and 1) abundance of questing adults and 2) intensity of larval ticks on infested hosts. Of 1,036 deer ticks collected, the vast majority (98%) were found only at the two largest sites. However, there was no significant difference among sites in the total abundance of small mammals (mean=0.25 per trap-night), suggesting that all the forested patches regardless of their size, had sufficiently abundant hosts to sustain tick populations. The small mammal fauna at the largest forest patch (n=69) was comprised mostly of *Peromyscus* (38%) and red-back voles, *Myodes gapperi* (58%). Although the prevalence of ectoparasitism for *Peromyscus* mice (88%) was significantly greater than for *M. gapperi* voles (60%), when engorged larval ticks were detached from the rodents and assayed for pathogens (i.e., xenodiagnoses), there were no significant differences in xenopositivity between infested *Peromyscus* mice and *M. gapperi* voles for either *Borrelia burgdorferi* (overall 6% xeno-positive animals) *or Anaplasma phagocytophilum* (overall 6% xeno-positive animals). Deer ticks have become established in discreet foci within northeastern North Dakota and the role of *M. gapperi* voles as reservoirs of Lyme disease and anaplasmosis in this region warrants closer scrutiny.

751

ENTEROBIUS VERMICULARIS: A FIVE YEAR EXPERIENCE FROM AN INNER CITY HOSPITAL IN THE BRONX

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Enterobius vermicularis (Pinworm or oxyuriasis) is an intestinal nematode infection commonly reported in school-age children in the United States. Reports of pinworm in the immigrant populations are emerging in the literature. Jacobi Medical Center, located in the Bronx, serves a large immigrant population. Clinical presentation, treatment and country of origin are described in patients with *E. vermicularis* infections presenting over a 5 year period to our institution. Twenty two patients were identified. Ten males (46%) were seen with mean age 17 (SD$\pm$ 17). Of these patients 20 (91%) were immigrants and of these, 6 (30%) patients gave a history of recent travel history to visit friends and family. The majority of patients seen were from Albania (9, 41%), other countries included Yemen (4, 18.2%), Morocco (4, 18.2%), Mexico (1, 5%), Pakistan (1, 5%) and Ecuador (1, 5%). Twenty patients (91%) were symptomatic. Pruritis ani was reported in 11 (50%) patients. Work-up was initiated for nonspecific abdominal pain in 8 (36%) patients and 4 (18%) patients were evaluated for enuresis. The scotch- tape was positive in 20 patients (91%). Of the 18 stools examined for ova and parasites, 4 (22%) revealed pinworm. Eighteen (82%)patients had other intestinal parasites detected on stool exam. *Dientamoeba fragilis* was found in 8 (36%) patients. The mean absolute eosinophil count was 0.5 (SD$\pm$ 0.5). Six (27%) patients had past history of pinworms which may represent recurrent infections, athough treatment failure cannot be excluded. Ten (50%) had a family members infected. Albendazole was administered to

our patients. The diagnosis is often missed or delayed because of the poor sensitivity of routine stool examination and a lack of awareness amongst primary care providers. Patients with *D. fragilis* identified in their stool should be screened for pinworm. Other members of the same household should be examined for pinworm. *E. vermicularis* needs to be considered in immigrant populations.

752

INTESTINAL PARASITES AMONG ACTIVE-DUTY MILITARY PERSONNEL IN THE PERUVIAN AMAZON

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Active-duty military personnel are exposed to soil-transmitted helminth (STH) infections when serving in highly endemic areas such as the Peruvian Amazon. Few studies have described the prevalence of STH and Strongyloides sterocoralis infection in this population. The aim of this study was to describe the prevalence of intestinal parasites among active-duty military personnel at the Peruvian Amazon basin. A descriptive observational study was carried out in a local army base of the Peruvian rainforest during December, 2012. We excluded individuals who received anthelminthic therapy in the last 3 months. One fresh stool sample was obtained from each participant and evaluated within 24 hours by the direct smear, spontaneous sedimentation in tube technique (SSTT) and modified Baermann's technique (MBT). Among 104 participants (age range, 15-34 years), 34% were infected by one parasite, whereas 57% had two or more. The most common helminths were hookworms (47%), S. stercoralis (29%), Ascaris lumbricoides (28%) and *Trichuris trichiura* (18%). The most common protozoa were *Blastocystis spp.* (57%) and *Giardia lamblia* (17%). The SSTT was the most sensitive test to detect helminthes and protozoa ($p<0.01$). MBT was more sensitive than SSTT to detect larvae of *S. stercoralis* ($p=0.04$). S. stercoralis was always associated with another parasite, and multiple regression analysis revealed a significant association to hookworm (OR = 3.88, 95% CI = 1.1-13.3), *A. lumbricoides* (OR = 3.98, 95% CI = 1.1-13.5) and *G. lamblia* (OR = 11.8, 95% CI = 1.59-86.8). We conclude that STH's and *S. stercoralis* are highly prevalent in active-duty military personnel serving in tropical areas and massive drug administration may be warranted in this high risk population. Furthermore, clinicians should consider the worldwide distribution of *S. stercoralis* before starting any immunosuppressive therapy in war veterans returning from endemic areas.

753

PREVALENCE OF *BLASTOCYSTIS SPP.* AND *STRONGYLOIDES STERCORALIS* AMONG MILITARY SOLDIERS FROM PERUVIAN RAINFOREST

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Blastocystis spp. is distributed worldwide and is the most frequent protozoa in human stools but is not commonly reported in field studies for its historically believed non-pathogenic role. A recent association between *Blastocystis spp.* and *Strongyloides stercoralis* has been suggested in a previous study from Japan. Despite school-aged children, others such as the military personnel on duty are also frequently affected by intestinal parasites. The aim of this study was to describe *Blastocystis spp.* and to determine its possible association with *Strongyloides* in military personnel of a local army of the Peruvian rainforest. A descriptive observational study was carried out in December 2012. One fresh stool sample from each participant was collected and evaluated by the direct smear, spontaneous sedimentation in tube technique and modified Baermann's technique. Data was analyzed by logistic regression to determine whether *Blastocystis spp.* was associated with *S. stercoralis* independently of other parasites. Among 104 individuals (age range: 15-34 years), the prevalence rate for *Blastocystis* spp was 54.8% (n=57) whereas the prevalence of *Strongyloides* was 28.8% (n=30). Two groups were analyzed separately, those with *Blastocystis spp.* versus those without this parasite in stools. The prevalence *of S. stercoralis* was higher in the group with *Blastocystis spp.* (70% vs. 30%) ($X2=3.92$, $p=0.04$). Logistic regression analysis confirmed the association between *Strongyloides stercoralis* and *Blastocystis spp.* (OR=3.1; CI=1.1-8.8; $p=0.03$), independently of the other parasites. We found an association between *Blastocystis spp.* and *S. stercoralis* in this military population, but the clinical importance and the underlying mechanisms of this association are unclear. These two parasites are highly endemic even in a non-school-aged population which constitutes another example of a neglected parasitic disease in the Amazonic region of Peru and further therapeutic interventions are needed.

754

ASSESSMENT OF THE EFFICACY OF ALBENDAZOLE FOR THE TREATMENT OF SOIL TRANSMITTED HELMINTHS IN EAST TIMOR

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Soil-transmitted helminths (STH), are among the most prevalent infections worldwide, and contribute to malnutrition and iron-deficiency anaemia, and adversely affect physical childhood growth. There has been a renewed global commitment to implement control strategies to reduce disease burden caused by STHs, particularly using regular periodic mass chemotherapy (MDA) with broad spectrum drugs such as Albendazole (ALB). The scale up of MDA will lead to increasing drug pressure on parasite populations, favoring the selection of drug-resistant parasites. In addition, the long term Public Health benefit of such MDA programs has been debated. The aim of this study, undertaken as part of randomised controlled trial looking at the impact of water, sanitation and hygiene (WASH) and ALB on STH infection, is to assess the antihelminthic efficacy of a single dose of ALB in communities in East Timor, where STH are endemic, by determining Faecal Egg Count Reduction (FECR) and Cure Rate (CR). Eligible community members (residents over one year of age and pregnant women in the first trimester) from 8 villages in Manufahi district were recruited. A total of 441 samples were collected immediately prior ALB distribution and 340 samples 7-12 days afterwards. All faecal samples were processed using a flotation technique for the detection and quantification of infections with STHs and protozoa. In addition, infection intensity was measured by multiplex qPCR. At baseline almost half of the participants were positive for *Ascaris lumbricoides* (44.8%), and hookworm (45.1%), while prevalence of T. trichiura was 1.1%. Very high cure rates were observed for *Ascaris*, while it was less efficacious against hookworms. While a single dose of ALB show high cure rates against STHs in Timor Leste, it will be important to monitor its efficacy over time, as the planned national MDA campaign is implemented. Furthermore, these results will have implications on the impact of the WASH intervention following ALB distribution on STH infection status; that is currently being trialed in the same area.

755

EPIDEMIOLOGY OF SOIL-TRANSMITTED HELMINTHS INFECTIONS AMONG ABORIGINAL SCHOOLCHILDREN IN RURAL MALAYSIA

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Despite the continuous efforts to improve the quality of life of Orang Asli (Aborigines) communities, the prevalence of soil-transmitted helminth (STH) infections among these populations remains largely unchanged since the 1920s, with alarming high prevalence rates and prominent morbidity. This study aimed at investigating the current prevalence and potential risk factors of STH infections among Aboriginal primary schoolchildren in the Lipis district of Pahang state, Malaysia. Faecal samples were collected from 468 children (52.1% males and 47.9% females) and examined by using formalin-ether sedimentation, Kato Katz and Harada Mori techniques. Demographic, socioeconomic, environmental and behavioural information were collected by using a pre-tested questionnaire. Overall, 96.6% of the children were found to be infected by at least one STH species. The prevalence of trichuriasis, ascariasis and hookworm infections were 95.7%, 45.9% and 24.6%, respectively. Almost two-thirds and half of the trichuriasis, and ascariasis, respectively, were of moderate-to-heavy intensities while all hookworm infections were of light intensity. Univariate and multivariate analyses showed that absence of a toilet in the house, using unsafe water supply as a source for drinking water, presence of other family member infected with STH, inadequate knowledge on STH, not washing hands before eating, and not washing hands after defecation were the significant risk factors of STH infections among these children. In conclusion, there is an urgent need to implement school-based deworming programmes and other control measures like providing a proper sanitation, as well as a treated drinking water supply and proper health education regarding good personal hygiene practices. Such integrated control programme will help significantly in reducing the prevalence and intensity of STH in Aboriginal communities.

756

LONG TERM EXPERIENCE WITH STRONGYLOIDOSIS IN SINGLE CENTER IN NORTHERN ITALY

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We conducted a retrospective study at the Infectious Diseases Clinic of the University Hospital IRCCS San Matteo (Pavia, Italy), aimed at the identification of strongyloidiasis cases diagnosed between January 1983 and December 2011. This led to the identification of 1013 patients, of whom we conducted an analysis of demographic, clinical and laboratory features, and response to treatment on a sub-cohort of 536, seen from 1998 to 2011. the prevalence of missing data was considered statistically acceptable (<20%). These patients were mostly elderly (mean age 68 years), males (60.3%) and autochthonous cases (92.2%). In most cases direct exposure to soil, the most likely risk factor, occurred during domestic activities. Diagnosis was based on identification of larvae in stool samples or positivity of immunological tests (2 ELISA commercial kits). At least one significant comorbidity was present in 55.6% of patients and 18% were receiving immunosuppressant therapy with steroids. Nonetheless, only 10 patients (1.9%) showed laboratory features suggestive of severe strongyloidiasis. Early recognition and treatment of the infection could account for this severe strongyloidiasis. Early recognition and treatment of the infection could account for this finding, but genetic variation may also be a rason. Marked eosinophilia (>9%) was present in 80% of cases at the time of diagnosis. Gastrointestinal symptoms and pruritus were the most frequent complaints, followed by cutaneous lesions and respiratory signs. We found a strong association between respiratory symptoms and comorbidity, as well as corticosteroid therapy ($p<0.001$), and gastrointestinal symptoms and comorbidity ($p=0.024$). Treatment with ivermectin appeared more effective than albendazole, considering the lower rates of positivity after therapy. Although the majority of cases could derive from remote exposure, the high incidence of new diagnosis and the few but unequivocal cases of reinfection suggest that screening at-risk population for stronyloidiasis should not be discontinued and a careful follow-up should be carried out.

757

COMPARISON OF KATO-KATZ AND MINI-FLOTAC FOR THE DIAGNOSIS OF SOIL-TRANSMITTED HELMINTHS: RESULTS FROM A FIELD STUDY IN THE REPUBLIC OF CONGO

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Soil-transmitted helminths (STH) are ubiquitous in the developing world where they affect the poorest and most deprived communities. The World Health Organization recommends the Kato-Katz (KK) method for detecting STH infections in the field. Disadvantages of KK are that it must be performed on fresh stool samples, and KK slides must be read promptly to avoid overclearing of hookworm eggs. We compared the sensitivity of KK with that of Mini-FLOTAC, a newly developed field method for STH diagnosis that can be used with either fresh or preserved stool samples. Three methods were used to analyse stool samples from 284 Congolese individuals aged 5 years and over. Two Kato-Katz smears were prepared for each individual. In addition, one gram of fresh material was used for the Mini-FLOTAC (Fresh-Flo); another gram of stool was preserved in 5% formalin solution and tested by Mini- FLOTAC (Pres-Flo) three months later. A*scaris lumbricoides* was detected in 54.6%, 53.9% and 50.0% of samples using KK, Fresh-Flo and Pres-Flo techniques, respectively, with intensities of infection (geometric mean of positive counts) of 5558, 2778 and 2543 eggs per gram (epg). *Trichuris trichiura* was detected in 79.2%, 69.7% and 70.1% of samples using KK, Fresh-Flo and Pres-Flo, respectively, with intensities of infection of 373, 179 and 146 epg. Hookworm was detected in 6.7%, 19.7% and 4.2% of samples using KK, Fresh-Flo and Pres-Flo, respectively, with intensities of infection of 41, 53, and 19 epg. Mini-FLOTAC performed with either fresh or preserved samples was not superior to KK for detection and intensity assessment of *A. lumbricoides* or *T. trichiura* infections. However, Mini- FLOTAC appears to be more sensitive than KK for detecting hookworm eggs in fresh stool samples than the other methods. This advantage was lost after samples were stored for 3 months. Pres-Flo has the potential to release stool testing from the time constraints of KK. Additional studies are needed to determine how long preserved stool samples can be stored before hookworm eggs are lost.

758

EFFICACY OF SINGLE-DOSE ALBENDAZOLE FOR SOIL-TRANSMITTED HELMINTH INFECTIONS IN PERUVIAN SCHOOLCHILDREN

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Soil-transmitted helminth (STH) infections contribute to the most disability-adjusted life years of all the Neglected Tropical Diseases, especially in school-age children, preschool-age children and women of reproductive age. To combat these infections, the World Health Organization recommends school-based, mass deworming programs in endemic areas along with continuous monitoring of drug efficacy in the community. The objective of the present study was to monitor the efficacy of single-dose albendazole for STH infection within the conduct of a school-based cluster randomized controlled trial in Peru. The study was conducted in Belén, in the state of Loreto, between April 21 and July 1, 2010. At baseline, stool specimens were collected from Grade 5 schoolchildren in 18 schools and analysed for STH prevalence and intensity using the Kato-Katz method. Following baseline assessment, all children were dewormed with single-dose albendazole (400 mg). Infected children were followed-up in schools two weeks following deworming and a second stool specimen was collected and analysed. A total of 385 children, infected with at least one STH species, participated in this follow-up study. The efficacy of albendazole was high for *Ascaris* (Egg Reduction Rate (ERR) = 98.8%; 95% confidence interval (CI): 92.1%, 99.9%) and hookworm infections (ERR = 86.3%; 95% CI: 71.6%, 93.0%) but much lower for *Trichuris* infection (ERR = 42.2%; 95% CI: 25.6%, 53.5%). These results are consistent with previous data published on the efficacy of albendazole. Efficacious preventive chemotherapy for *Trichuris* infections continues to be a challenge. Innovative research, including a back-to-biology-basics approach, to fully understanding *Trichuris* microepidemiology, may provide additional insight.

759

THE *NA*-GST-1 HOOKWORM VACCINE IS SAFE AND INDUCES NEUTRALIZING ANTIBODIES IN BRAZILIAN ADULTS

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Necator americanus glutathione S-transferase-1 (*Na*-GST-1) is a 24-kDa protein that catalyzes the conjugation of reduced glutathione to a variety of electrophiles. *Na*-GST-1 belongs to a class of nematode GSTs characterized by diminished peroxidase activity relative to other GSTs but increased binding capacity for heme and related products. It is produced by adult hookworms and is thought to play a role in detoxifying heme and other breakdown products of the hookworm blood digestion pathway. Vaccination of laboratory dogs and hamsters with recombinant GST-1 results in reduced hookworm fecal egg counts and adult worm burden following challenge with infective larvae. Recombinant *Na*-GST-1 was expressed in *Pichia pastoris* and formulated with Alhydrogel. 36 healthy Brazilian adults with no history of hookworm exposure and living in the city of Belo Horizonte were enrolled in a dose escalation Phase 1 clinical trial of *Na*-GST-1. Volunteers received 1 of 3 different dose concentrations of *Na*-GST-1 (10, 30 or 100 µg) in 1 of 2 different formulations (*Na*-GST-1/Alhydrogel or *Na*-GST-1/Alhydrogel to which 2.5 µg of the Toll-like receptor-4 agonist, glucopyranosyl lipid A [GLA-AF], was added as a point-of-injection preparation). Subjects received 3 intramuscular injections at 2-month intervals. Subjects were screened by ELISA for serum IgE antibodies to *Na*-GST-1 due to the previous experience with IgE-related urticarial reactions induced by the recombinant *Na*-ASP-2 hookworm vaccine; all were negative. Common vaccine-related solicited adverse events included mild to moderate injection site pain and tenderness (2 cases of severe pain), headache (1 severe), and nausea; no differences between dose groups were observed in incidence of adverse events. Anti-*Na*-GST-1 IgG antibody levels as measured by ELISA were modest after the 2nd vaccination, but increased significantly from baseline after the 3rd vaccination in those who received 100 µg *Na*-GST-1. For each dose concentration of *Na*-GST-1, the increase in IgG levels was not significantly different in those who received formulations containing GLA-AF. Importantly, induced IgG antibodies inhibited binding of heme by *Na*-GST-1 in an *in vitro* assay. These data demonstrate that *Na*-GST-1/Alhydrogel is well tolerated and induces anti-*Na*-GST-1 IgG antibodies that have functional activity in inhibiting the target antigen. Further testing in hookworm endemic populations is on going.

760

IMPACT OF PERIODIC SELECTIVE MEBENDAZOLE TREATMENT ON SOIL-TRANSMITTED HELMINTH INFECTIONS IN CUBAN SCHOOLCHILDREN

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Periodic treatment with 500 mg mebendazole is one of the strategies currently recommended by the WHO to control soil-transmitted helminth (STH) infections in endemic areas. The efficacy of anthelminthic drugs has been investigated within randomized controlled trials (RCTs). Various studies exist on the effectiveness of periodic anthelminthic treatment, but mainly in the context of (targeted) mass treatment studies. Here, we evaluated the impact of periodic selective treatment with mebendazole on STH infections in Cuban schoolchildren. We followed up a cohort of 268 STH-positive schoolchildren -aged 5-14 years at baseline- at six months intervals for two years and a final follow-up after three years. The Kato-Katz stool examination technique was used to detect infections with *Ascaris lumbricoides*, *Trichura trichiura*, and hookworm. Common risk factors related to STHs were assessed by parental questionnaire. A significant reduction in the number of STH infections was obtained after three years with the highest reduction for *T. trichiura* (87.8%) and the lowest for hookworm (57.9%). After six months cure rates (CRs) were 76.9% for *A. lumbricoides*, 67.4% for *T. trichiura*, and 44.4% for hookworm. After two treatment rounds, more than 75% of all STH positive children at baseline were cured, but with important differences between STH species (95.2% for *A. lumbricoides*, 80.5% for *T. trichiura*, and 76.5% for hookworm). At the end of the study, these cumulative CRs were almost 100% for all three STHs. Risk factors for STHs were sex, sanitary disposal, and habit of playing in the soil. Our results indicate that periodic selective treatment with a single dose of 500 mg mebendazole is effective in reducing the number of STH infections in Cuban schoolchildren. Although important differences were found between helminth species, two rounds of treatment appeared sufficient to obtain substantial reductions.

761

THE IMMUNOLOGICAL RESPONSE OF HIV POSITIVE PATIENTS INITIATING HAART AT THE KOMFO ANOKYE TEACHING HOSPITAL, KUMASI, GHANA

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Highly active antiretroviral therapy (HAART) is the mainstay of treatment for people living with HIV (PLHIV). Although there is enough documented evidence on immunological response of patients to HAART elsewhere, in Ghana, it is not well documented. We document the experience of immunological improvement among Ghanaian PLHIVs on HAART, comparing different categories of patients. Questionnaires were used for patient demographic and clinical data. Four CD4 counts were measured at six-monthly intervals to determine and compare rates of CD4 change among different categories of patients. Women had higher CD4 count (77.4cells/µl) at baseline. CD4 count increased from a mean baseline of 70.2 cells/µl to 229.2, 270.0, and 297.6 cells/µl at 6, 12, and 18 months of treatment respectively ($p < 0.0001$). There were no gender ($p=0.46$) and age ($p=0.96$) differences in treatment response. There was no difference ($p=0.18$) in treatment response comparing patients with CD4 <250 cells/µl and those with CD4 count between 250-350 cells/µl. Out of 282 patients with pre-therapy CD4 count ≤250 cells/µl, 241 (85.5%) and 41 (14.5%) were adherents and non-adherents respectively. Mean rate of increase was 15.2 and 8.4 cells/µl/month in adherents and non-adherents respectively ($p=0.2$). The findings of this study suggests that a sustained CD4 increase could be achieved in adherent patients commencing therapy with baseline CD4 count <250 cells/µl. These patients have greater ability for immunological recovery during 12 months of treatment. We, therefore, conclude that significant immunological improvement is therefore possible among Ghanaian PLHIV on HAART as long as a high level of adherence is observed.

762

HIV COUNSELING AND TESTING AMONG PATIENTS WITH TUBERCULOSIS AT ARBAMINCH HOSPITAL, SOUTHERN ETHIOPIA

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Ethiopian National tuberculosis (TB) and Human Immune Virus (HIV) guideline that recommend HIV counseling and testing as part of routine TB care was in effect since 2005. However, the number of TB patients that know their HIV status remains low in the country. The objective of our study was to assess the HIV counseling and testing among TB patients at Arbaminch Hospital, Southern Ethiopia. We conducted a cross sectional study from January to April 2012 at Arbaminch Hospital (AMH). Newly diagnosed TB patients who fulfilled the inclusion criteria were enrolled for this study. The sample size was calculated using a single proportion formula and participants were recruited sequentially. Socio-demographic and TB/HIV related information of study participants were collected using pre-tested interviewer administered questionnaire. The HIV status and other clinical data of study participants were taken from the TB treatment registration book in the TB clinic. We enrolled a total of 76 newly diagnosed TB patients. The majority of study participants (92.1%) reported that they have been consulted by physician to take an HIV test when they were diagnosed with TB. Among study participants consulted by physician to get HIV testing, 54.3% did not receive counseling service. One-fourth of patients who received the counseling service did not go on to get tested. Overall, 23.7% of the study participants were receiving anti-TB treatments without their HIV status determined. None of patient related factors we assessed were associated with obtaining consultation and counseling services and with willingness to get tested except for residence ($p=0.041$) and previous HIV screening history ($p=0.004$). In conclusion, the HIV counseling and testing service given to TB patients in the Hospital was low and poorly harmonized which calls for alternative strategies to improve willingness of TB patients to be tested; and meliorating awareness of physicians on the benefits of the testing. It also sounds like improving the coordination between physicians and counselors is vital if providing consultation, counseling and testing services in one setting is not possible.

763

THE EFFECTS OF MALARIA AND HIV CO-INFECTION ON HEMOGLOBIN LEVELS IN PREGNANT WOMEN IN SEKONDI-TAKORADI METROPOLIS, GHANA

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This study was undertaken to assess the burden of malaria and human immunodeficiency virus (HIV) co-infection and to determine the risk of anemia among dually infected pregnant women in Sekondi-Takoradi, Ghana. A cross-sectional study was conducted at four hospitals in the Sekondi-Takoradi metropolis comprising 872 consenting pregnant women attending their antenatal care clinics, cross-checked with ultra sound or with clinical evidence of pregnancy. The study showed that 34.4% of the pregnant women had anemia while 65.6% were non-anemic. Multivariable logistic regression analysis indicated that pregnant women with a single infection with either malaria or HIV were independently associated with increased odds of maternal anemia. In adjusted models, pregnant women co-infected with malaria and HIV doubled their risk of maternal anemia (adjusted OR, 2.67, 95% CI, 1.44-4.97, P = 0.002). In conclusion, dually infected pregnant women with malaria and HIV are twice likely to be anemic than those with a single or no infection. For all pregnant women in this region, it is imperative to control for malaria, HIV and anemia in other to improve birth outcomes.

764

MOLECULAR TYPING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM RESPIRATORY TRACT OF HIV-POSITIVE CHILDREN AND ADOLESCENTS IN CAMBODIA

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HIV-infection is an important risk factor for acquisition of methicillin-resistant *Staphylococcus aureus* (MRSA)-associated infection. Molecular typing methods are useful for study of relationships of outbreak-causing strains in health-care settings, but many of them are time-consuming, expensive and require experienced stuff. In this study we aimed to assess incidence of MRSA in group of HIV-positive Cambodian children and adolescents and to evaluate applicability of HVR-mecA-typing for detection of clonal MRSA spread. Samples from HIV-positive patients (n=42) living in two long-term health care facilities for orphans in Cambodia (Phnom Penh and Sihanoukwille, respectively) were included. Bacteria retrieved from respiratory swabs in 2006 and 2012 were analyzed. Altogether, 156 isolates were obtained, of which 56 (35,9 %) were identified as S. aureus and 29 (18,6 %) of these were MRSA. Eighteen (n=18) MRSA isolates recovered from stock cultures were included for further typing of hypervariable region of mecA gene (HVR-mecA-typing) to assess polymorphisms in direct repetitive units (DRU). HVR-mecA-typing revealed six different HVR-types, with HVR-type I (fragment length 575 bp) being the most prevalent (41,2 %), followed by types II and V (17,5 % both). Considering resistance, except of methicillin resistance, erytromycin resistance was the most frequent (88,9%), followed by clindamycin (61,1%) and cefuroxim (61,1 %) resistance. No correlation between

resistance pattern and HVR-type was observed. Interestingly, respiratory tract infections, but not skin and soft tissues infections, were the most common in patients with MRSA. According to typing results we assumed evolution out of one or two different SCCmec resources. As the HVR-types are a lot heterogenous, we suppose that the spread of MRSA in those particular facilities is not clonal, but the HVR has diverged overtime. This is interesting, considering fact that in health care settings MRSA are usually spread clonally.

765

FIRST INDIRECT MEASURE OF INCIDENCE AND RISK FACTORS FOR RECENT INFECTIONS WITH HIV-1 AMONG FEMALE SEX WORKERS IN THE DISTRICT OF BAMAKO, MALI

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In Mali, HIV is a public health problem with an overall prevalence of 1.3% in the general population. This prevalence reaches 26% in some risk groups such as female sex workers. The incidence of HIV, an important element in assessing the impact of control interventions, has never been evaluated in Mali. The recent settling of HIV incidence tests and the UNAIDS "vision three zero" have paved the way for indirect measures of HIV incidence among vulnerable populations. We conducted a cross-sectional survey involving 388 sex workers in Bamako who underwent interview, diagnostic tests (HIV-1/2) and incidence tests of HIV-1 with the BED-EIA (Enzyme Immuno Assay) to identify recent infections in women infected with HIV. The socio demographic characteristics, the factors related to health services and those related to the practice of sex work were taken into account in a multinomial logistic regression model to assess the associated risk for a recent infection as compared to the non-infected. Among the 388 sex workers included with a median age of 25 years (18-53), 71 (18.3%) were seropositive to HIV-1. Based on the adjustment method of Kassanjee *et al*, the incidence was high with [8.84%; 95% CI (4.21 to 13.46)]. The age 35 and more , the number of years worked as a sex worker, the number of customers per day greater than 5 were the main risk factors for recent HIV-1 infection with respectively [OR = 5.94 (1.03 to 34.15)], [OR = 1.18 (1.01 to 1.39)] and [OR = 17.87 (1.87 to 208.92)] at 95% CI. The participation to a VIH screening test less than a year ago, the interaction between education status and the number of customers per day greater than 5 were protective factors against a new HIV infection with respectively [OR = 0.25 (0.07 - 0.87)] and [OR = 0.03 (0 - 0.41)] at 95% CI. The overall incidence of HIV-1 infection was high among sex workers in the district of Bamako in 2012. The development of professional reorientation programs of sex workers in the context of improving the HIV prevention activities in certain risk groups may help reduce this incidence.

766

THE DISTRIBUTION PATTERN OF SEXUALLY TRANSMITTED INFECTIONS AMONG FEMALE CLIENTS VISITING FOR MEDICAL LABORATORY TESTS IN LAGOS, NIGERIA

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Eight hundred and seventy-nine female clients reporting for medical Laboratory diagnosis were screened in a medical diagnostic laboratory in Lagos, Nigeria. Blood sample was collected from 246 (28.0%) clients for serological screenings, while urine and High vagina swab (H/V/S) samples were collected from 633 (72.0%) for microbiological analysis and culture. The women were of child bearing age with range between 15 - 42 years old. 332(37.8%) of the clients were not married while 547(62.2%) were married women. The mean age of the un-married women was 26.7 (±3.25 SD) while married was 33.80 years (±10.35 SD). There was no difference in ages (p-value = 0.4). The group age of the participants include 15 - 20; 21- 25; 26 - 30; 31- 35; 36- 40 and 41+. Overall, urinary tract infection (UTI) caused by bacteriological organisms was top in the list of infections 357/879(40.6%) + 16(1.8%) followed by HIV types 1 and 11 91/879 (10.4%); Candidiasis 48/879(5.5%); while Syphilis, genital warts and hepatitis B. Infection were 2(0.2%) each. Married women were more infected with UTI 214/357(60.0%) compared to unmarried 143/357(40.0%), infection was statistically significant with p-value =0.1. Similarly more married women were more infected with HIV 54/91(59.3%) compared to unmarried 37/91(40.7%). Infection varies and increases with age, among the married UTI was more with the 26-30 age group 90/214(42.0%) while among the unmarried it was more with the 21- 25 age group 67/143 (46.8%). HIV infection was more with the 31- 35 group age among the married 32/54(59.2%) while among the unmarried it was more with the 21- 25 age group 16/37 (43.2%). More married women were infected with candidiasis 32/48(66.7%) with the 26-30 age group 9/32(28.1%) topping the others compared to unmarried 16/48(33.3%) with more infection among the 21- 25 age group 10/16(62.5%). High prevalence rate of sexually transmitted infections is a public health risk. Clinicians and program managers should promote routine screening of all pregnant women for infections and early treatment for both married and unmarried population while providing community health education to reduce spread.

767

BARRIERS TO UTILIZATION OF PROVIDER-INITIATED HIV COUNSELING AND TESTING SERVICES AMONG TUBERCULOSIS PATIENTS: A CASE OF RHODES CHEST CLINIC NAIROBI, KENYA

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Tuberculosis (TB) continues to be one of the most important global public health threats. HIV prevalence among TB patients in Sub-Saharan Africa is 70%.In Kenya, over 60% of TB patients are HIV positive(MOH,2007). The objective of the study was to determine the barriers to utilization of provider initiated HIV counseling and testing services among TB patients. A cross sectional survey of TB suspects visiting chest clinic was conducted. Consenting patients who visited the clinics during October to December 2010 were the study subjects. Data was collected through structured interviews with TB patients visiting the facility using a standard questionnaire and direct observation. The quantitative data was analyzed using descriptive statistics. A chi square test was used to interpret results for each possible barrier in terms of utilized versus declined to utilize HIV counseling and testing services. The test was considered to be statistically significant if the P-value was < 0.05. It was found that 83 % of TB patients tested for HIV infection. The main reasons for not being tested were that they don't trust confidentiality (17.9%), fear of positive test results (11.9%), fear of discrimination (10.4%), fear of being stigmatized (9.0%) and self perception of low risk (7.5). (χ2=29.473, 9 df, p=0.030). Factors that were significantly associated with utilization of PITC services were level of education (χ2=116.045,2df,p=0.0001), HIV stigma (χ2=36.947,3df,p=0.0001), awareness of HIV-TB link (χ2=22.767,2df,p=0.0001) and discussion of HIV/ TB link by nurse (χ2=59.232,2df,p=0.0001). In conclusion, TB patients evidently experienced both patient related and provider based barriers.The NACC, NLTP and TB/HIV Partners should scale up community awareness about HIV-TB co infection and train all providers on collaborative HIV-TB services. Advocacy for HIV screening for all TB patients should also be increased.

768

ADVERSE NEUROLOGICAL EVENTS DUE TO ANTIRETROVIRAL THERAPY IN MALI

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Adverse neurological events during antiretroviral treatment (ART) are frequent and various. Their diagnosis occur some difficulties in different orders according to the geographical context. To identify the frequency of neurological side effect, we performed prospective study taking care of any patient under antiretroviral treatment and developing neurological manifestations in a period of 12 month in infectious diseases service located at the teaching hospital "Point G", Bamako, Mali. Neurological diagnoses have been done with the guidance of a neurologist. All data were collected in a file. Side effects classification according to WHO has been used to characterize them[4]. Analysis of data has been done in the Software SPSS version 12.0. Four hundred and twenty two (420) HIV seropositive patients under ART treatment have been followed. Among them, 37 cases have been discovered with adverse neurological events (8.08%). The sex ratio M/F was 1.06. The age average was 41.2 years. Polyneuritis alone represented 83.8%, and then Polyneuritis associated to vertigo, headache and depression represented 16.2 %. We didn't notify any neurologic symptoms at the ART initiation. The major part was infected by HIV-1(91.9 %). 89.2% of them were under fixe dose combination Triomune® (D4T+3TC +Nevirapine). Five cases were at 3rd stage of WHO classification (13.5%) what justified consequently the stopping of d4T. Nevertheless, adverse neurological events may arise by using Triomune®. In future antiretroviral therapy must take into account neurological consequences and the instauration of pharmacovigilance to detect eventual drugs with neurological side effect.

769

RELATIVE LOW NUMBER OF NEW HIV CASES DETECTED IN RURAL DISTRICT BUNDA IN NORTHWEST TANZANIA

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HIV prevalence in East Africa is still of concerns and was initially 15% - 25 % in Uganda, Kenya and Tanzania in 1995 - 2005. However, it decreased gradually to 10% - 20% after introduction of ABC educational campaign, point-of-care testing during outbreaks in 2010, mother-to-child-transmission prevention (MTCTP) since 2003 and increasing number of treated patients and after 2005 global Fund and world Bank introduced free generic antiretroviral payment (e.g. NASCOP) in Kenya. The aim of this study was to determine HIV occurrence in newly tested individuals in distinct hospital voluntary counselling and testing (VCT) and AIDS cases in Bunda, Tanzania. Altogether 14499 patients came at study period to outpatient department (OPD) and inpatient (IPD) units (general, internal unit, paediatric, surgical, and maternity units). These were treated since July 2011 to August 2012, with an average of 1200 - 1300 patients a month (674 - 789 OPD and 363 - 514 IPD). Incidence of new HIV-positive cases, malaria, tuberculosis cases, pneumonia and diarrhoea as well as sexually transmitted diseases (STD) were monthly assessed. Within last 5 years, 1194 new cases of HIV were detected (in 2005 - 2010) and 226 (19%) of these patients receive antiretroviral therapy (ARV), which counts for approximately 20 new treated cases per month (240 a year). Of 1037 - 1210 monthly visits, about 400 - 500 were tested, which is 3 - 4% prevalence, even in sick patients population. Since July 2011, monthly prevalence was 10-15 cases/1000 -1200 patients (1 - 1,5%), which is much less than prevalence reported by government in West Tanzania. Low prevalence of HIV positive patients in Kibara Hospital is constantly decreasing from 8 - 15% as reported by WHO to 3 - 4% in 2005 - 2010 and even to 1,5-3% in 2011/2012. This is probably due to active outreaching and screening policy, access to VCT, free ARV, testing and ABC educational campaign in Tanzania.

770

ROLE OF CARE AND TREATMENT CENTERS ON INFLUENCING ADHERENCE TO ANTIRETROVIRAL THERAPY BY HIV PATIENTS IN DAR ES SALAAM

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HIV care and treatment centres (CTC) are being established to support HIV patients to live a healthy life. It is important to investigate how CTC attendance relates to patient adherence to antiretroviral therapy (ART) and ultimately influence treatment response. For this reason, the patients' adherence to ART, and associated factors were correlated and the role of CTC in influencing this was evaluated. This was an analytical cross-sectional study conducted in Dar es Salaam. Data abstraction was done using structured questionnaires and records review. Descriptive analysis was done for the demographic and clinical characteristics. The chi-square and multivariate logistic regression analysis were used to identify factors related to adherence. Four hundred and twelve patients attending CTC in Dar-Es-Salaam were recruited. There were 134 (33%) males with median age of 42 years. Sixty five (16%) had post primary school education, 121 (29%) had no stable income and 98 (24%) had an income of more than 100 USD per month. Two hundred and nine (51%) were self-employed, 138 (33%) were unemployed. Three hundred and two (73%) have not been on care before starting ART and 316 (77%) had been on ART for more than 12 months. Two hundred and fifty two (61%) reported side effects while on ART and 233 (57%) perceived care at CTC as very good. The prevalence of adherence based on consistency of keeping appointments was 314 (76%), based on three days recall was 362 (88%) and based on taking ARV more than 90% of the time was 234 (57%). Our findings consistently found high adherence prevalence based on three days recall and consistency of keeping appointments. The key factors affecting adherence were; missing appointment and registering late to CTC. We found that provision of adequate education on the importance of strict adherence to the prescribed doses of ARVs during regular attendance to CTCs is an important factor towards achieving good adherence to ART. Future research should explore what factors in rural setting in Tanzania presents barriers to adherence.

771

HUMAN AFRICAN TRYPANOSOMIASIS UNCERTAINTY IN GHANA

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Some countries in the West African Region, including Burkina Faso and Cote d'Ivoire have reported cases of Human African Trypanosomiasis (HAT) in the past decade. Ghana, after the control of the HAT epidemic in the 1940's, has not had active surveillance for the disease. There have however

been sporadic reports of HAT in the last decade, especially, in the Western Region of Ghana. HAT is not seen to be of public health concern in Ghana thus not considered by doctors in major hospitals as cause of sickness in patients reporting to health centres although tsetse flies, the vectors of the disease, occur in many parts of the country. Symptoms of the disease are similar to diseases such as malaria and common flu, making the situation a serious one as when left untreated, patients become reservoirs. This study aims to identify species of trypanosomes in naturally infected flies, using pig farms in the Eastern Region, Ghana, as a case study. Pigs and other domesticated ruminants have been incriminated as reservoirs for *Trypanosoma brucei gambiense*, causative agent of HAT. The region has in past years recorded cases of trypanosomiasis in pigs with farmers incurring up to 50% losses within 2006/2007. The Eastern Region is located in the southern sector of Ghana and is mostly forest vegetation. *Glossina palpalis*, vector for the HAT, is the dominant tsetse species in that region. Biconical traps were set close to sties in five pig farms and trapped tsetse flies were morphologically identified and sorted then, non teneral flies were dissected to collect mid guts, hypo pharynx and salivary glands. Molecular analyses of the samples are on- going to determine the tsetse flies' sources of blood meal and the Trypanosoma species they may be infected with. This process is expected to be completed by mid- April. Results and data will be presented. Results from the study are expected to shed more light on the situation of HAT in Ghana by revealing the species of trypanosomes circulating in the system; examine the pattern of transmission and determine chances of an outbreak of HAT in within that area. This will inform the appropriate monitoring and surveillance methods to curb possible future epidemics.

772

THE HUMAN AFRICAN TRYPANOSOMIASIS (HAT) IN SENEGAL: MULTIDICIPLINARY APPROACH FOR AN IDENTIFICATION OF THE AREAS AT RISK

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Today the trypanosomiasis always represents public health problems in some countries of sub-Saharan Africa, even if it is recognized in a general way that the situation clearly improved following the example of West Africa. However the situation under area remains fuzzy since very little, even not of information are available for 6 countries, as Senegal which formerly sheltered active hearths of trypanosomiasis. Besides many areas of West Africa were not visited since independences. In Senegal the history of the HAT goes back to the colonial period. The first cases of trypanosomiasis were announced on the Small Coast by Sice which describes them under the name of "Nélawane". Many cases were observed of 1900 the day before independences, in the areas contaminated like the Delta of Senegal, Niayes, the Small Coast, Casamance and Eastern Senegal. The disease had almost disappeared in the Sixties, at which time she was then regarded as residual. The last case recorded in Senegal goes back to 1977 in a 18 year old young man born in Mbour an old active hearth. Since then, no case of disease of the sleep was announced, but also no program of epidemiologic monitoring was set up. Only a project of fight against the glossines in Niayes and part of small the east coast in the course of execution. However after having revisited the principal historical hearths of Senegal, we studied the distribution and the densities of glossines via the degree of development of hydraulic works as well as the hydrographic network and pluviometry in the areas formerly contaminated by the HAT. The analysis of these various factors besides the migratory bond with the infected countries made it possible to identify the areas at the risk of HAT namely: the Low one and High Casamance, the mouth of Sine Saloum, the zone of Kédougou and Niayes. Thus a reactualization of the epidemiologic and entomological data is essential.

773

EVALUATING THE INTERRUPTION OF *TRYPANOSOMA CRUZI* TRANSMISSION IN COMMUNITIES WITH REEMERGING VECTOR POPULATIONS

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In order to assess the effectiveness and achievements of vector control programs for Chagas disease, evaluation goals and accompanying procedural guidelines have been defined. However, as some programs struggle with the concurrent challenges of persistent reinfestation, instituting and maintaining robust vector surveillance programs and sustaining interest and funding for vector control activities, the need to reassess and strengthen evaluation procedures has become increasingly relevant. One particular concern in the context of evaluating Chagas disease control programs is how best to optimize the selection of indicators for evaluation and their application in the evaluation process. Drawing on data from the published literature and reports produced by national and international institutions, we offer a review of the evaluation process for Chagas disease vector control programs. First, we describe the principles and existing evaluation process for these programs. Next, we review the historical origins of the use of seroprevalence in children between 0 and 5 years old as a primary indicator for evaluation. We then examine the alternative indicators to assess progress toward interruption of transmission and conduct a critical review of the current recommended evaluation procedures. We identify seven key indicators that may be used to evaluate Chagas disease vector control programs. The origin and historical application of these indicators are further examined, along with the advantages and weaknesses associated with their use in the evaluation process. In addition, we offer strategies for strengthening the evaluation process by identifying several key areas for improvement in the current evaluation process, including the importance of using a complement of indicators to measure progress toward interruption of parasite transmission and the need for more stringent adherence to periodic surveillance after certification.

774

SCREENING OF POTENTIAL ANTI-*LEISHMANIA*L DRUGS AGAINST INTRACELLULAR *LEISHMANIA* BY A HIGH-THROUGHPUT DRUG SCREENING FORMAT

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Leishmaniasis is caused by protozoan parasites of the genus *Leishmania*. The disease is highly endemic in tropical and sub tropical areas and the Mediterranean basin, threatening around 350 million people worldwide. The parasite is transmitted to the mammalian host by the bite of phlebotomine sand flies. There are three dominant forms of clinical leishmaniasis. The most common form is cutaneous leishmaniasis usually presenting as solitary ulcer. Muccocutaneous leishmaniasis can cause disfiguring skin lesions. The most severe form is visceral leishmaniasis, where the parasite migrates to liver, spleen and other organs. If untreated, death may occur. There is still no vaccine protection against leishmaniasis, so chemotherapy is critical. Only two drugs sodium stibogluconate and amphotericin B are in currently used widely. They have several side effects. There is, therefore a need for new cost-effective drugs for the treatment of leishmaniasis. Primary drug screening is often apprised out with the promastigote stage of parasite since it is easy to maintain in the laboratory. But an anti-leishmanial drug should be tested against the intracellular form or in amastigote. We have developed a host cell based drug screening assay using a human macrophage cell line infected with the causative

agent of visceral leishmaniasis, *Lesihmania donovani*. This assay format directly screens compounds against the intracellular form of *Leishmania*. We are currently collaborating with different institutes, companies and universities to screen thousands of compounds against the intracellular parasite. Results of these screens will be presented.

775

WHOLE BLOOD IFN-Γ RELEASE ASSAY IN IDENTIFYING EXPOSURE TO *LEISHMANIA DONOVANI* INFECTION IN HIGHLY ENDEMIC VILLAGES IN BIHAR, INDIA

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Of all persons infected with the parasites causing visceral leishmaniasis (VL) usually only 10-25% progress to clinical disease. We have recently developed a whole blood IFN-γ release assay (IGRA) as a marker of cellular immunity to detect infected and non- infected persons with high accuracy. In the present study, we employed this test along with quantitative PCR (qPCR) amongst healthy individuals living in the kala-azar endemic zone in Bihar, India for identification of individuals exposed to leishmania infection but without disease. We enrolled 13,163 persons from eleven highly endemic villages to identify the incident infected healthy persons as measured by seroconversion with DAT and rK39 ELISA at base line survey and at 12 months interval. Whole blood assay and qPCR were performed longitudinally for two years amongst seropositive and its matched seronegative controls populations. Level of IFN-γ was determined in antigen stimulated whole blood culture supernatant by conventional ELISA. Subjects were followed up on monthly basis to monitor the progression into disease. Of 13,163 persons only 309 subjects (3.6%) had converted to seropositive on either of the DAT or rK39 ELISA in one year interval. The percentage positivity of WBA and qPCR was equal in both seropositive (IGRA =19%, qPCR= 48.2%) and its control groups (IGRA =16.3%; qPCR= 42.8%) (p= 0.431; $\chi 2$ test). Of those IFN-γ positive persons, 63.8% and 60.7% persons remained IFN-γ positive over 1 year in seropositive and control groups respectively. The new incidence of IFN-γ positivity was 5.5%. Only one subject who was positive by both IGRA and qPCR at baseline developed into clinical VL. These findings confirm that SLA based IGRA is a promising tool for identification of clinically exposed immune individuals.

776

DETECTION OF *LEISHMANIA DONOVANI* AND *LEISHMANIA MAJOR* IN NATURALLY INFECTED SAND FLIES IN ISIOLO AND WAJIR COUNTIES, KENYA

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Leishmaniasis has been termed as an emerging or re emerging disease in North Eastern Kenya. Over the past decade, several out breaks have been reported In Isiolo and wajir. While the causative agent, *Leishmania donovani*, has been isolated from the human so far, no work has been done to identify possible invertebrate hosts. Previous studies have pointed at *Phlebotomus orientalis* as the possible VL vector. However, natural infections have not yet been demonstrated. This study was designed to test sand flies from the area for natural *Leishmania* infection. Sampling was done between 2008-2011 in areas both in Isiolo and Wajir using CDC light traps baited with dry ice. Sand fly caught were stored in 70% ethanol and transported to the laboratory for processing. Ten percent of the collection was used for species identification while the rest (females) were pooled for DNA extraction and conventional PCR for detecting *Leishmania* infection (genus assay) and real time PCR for identifying the specific *Leishmania* species (species assay).A total of 1486 pools from Wajir and 2465 from Isiolo were tested, giving a total of 14078 and 30913 females respectively. Two pools from Wajir and three from isiolo tested positive for *L. donovani* while three additional pools from Isiolo tested positive for *L. major.* The minimum infection rates were .13% in wajir and 0.24% in Isiolo.The first report of naturally infected *L. donovani* and *L. major* in sand flies collected from both counties. The finding of *L. donovani* infected sand flies indicates that there is potential transmission of both Cuteneous leishmania (CL) and Visceral leishmania (VL) in the region. The finding of *L. major* positive sand flies raises questions on whether there are other vectors of *L. major* in Kenya apart from *P. duboscqi.*There is need for further investigation on the vector competence of the sand fly species of Isiolo and Wajir as well as a correlation with infection in human. Need for active surveillance in the region in light of political and civil unrest in neighboring *Leishmania* endemic countries.

777

HEALTHY HOUSING FOR HEALTHY LIVING: PARTICIPATORY DESIGN OF A HOUSING PROTOTYPE TO CONTROL VECTORIAL TRANSMISSION OF CHAGAS DISEASE IN LOJA PROVINCE, ECUADOR

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Chagas disease is caused by the parasite *Trypanosoma cruzi*, mainly transmitted by the feces of triatomine insects. Considering that triatomines find favorable environments in cracks of walls, floors, and roofs of poorly constructed houses, Chagas disease is deeply connected with life conditions present in rural areas of Latin America. Consequently and based on ten years of entomological research that shows constant triatomine reinfestation in the houses of Loja province in southern Ecuador, despite insecticide and educational interventions. We developed a *Healthy Housing Model* that considers the physical structure, disposition and organization of intra and peri-domicile as the main tool for long term Chagas disease control in the region. In order to guarantee social and cultural acceptability of the model, as well as communities' participation in the different phases of the project from decision-making to implementation and evaluation, we implemented a participatory research process based on positive deviance (PD) methodological framework. This process was aimed at identifying existing practices and knowledge in relation to houses' construction and insect protection, as well as attitudes in relation to locally available construction materials and improvement techniques. The descriptions and traditional knowledge shared through interviews, photo-voice and participatory sketching exercises, as well as housing infrastructure data collected from all the families were used to design a prototype that was designed with the communities in multiple sessions and adjusted by the architecture team to local communities' practices and aesthetics. Three different models of the prototype were developed and construction training was provided to community members to ensure technological transfer of the improvements implemented in the housing prototype. The prototype was constructed as a joint effort between the community and researchers, to serve as proof of concept prior to the possible scaling up of the intervention at the whole community.

778

AN EPIDEMIOLOGICAL MAP OF CUTANEOUS LEISHMANIASIS IN THE KINGDOM OF SAUDI ARABIA

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Cutaneous leishmaniasis (CL) is the predominant vector-borne disease in the Kingdom of Saudi Arabia (KSA). Despite a significant reduction in the number of cases in recent years due to effective vector and rodent control initiatives, the uncontrollable urbanisation and the growing number of unresponsive patients to drug therapy are major concerns. Both *Leishmania major* and *L. tropica* are responsible for all CL cases in KSA, but their geographical distributions are unknown as patients are only clinically diagnosed. In this work, we report the molecular characterisation of the *Leishmania* spp from the main CL endemic regions in KSA. In addition, we describe the clinical features of the cutaneous lesions associated with each parasite species and patient responses to standardised drug treatments. Samples from a total of 104 adult individuals (89% male, 11% female) were collected from the regions of Riyadh, Algassem, Asir, Jazan, Al Ahsa, Al Madinah and Hail, which collectively represent ~80% of all reported cases of CL in KSA. Wound aspirations and skin biopsies were collected to identify parasite species and secondary infections, respectively. In addition, serum samples were taken to confirm leishmaniasis diagnosis using a novel ELISA method that measures levels of anti-alpha-galactosyl antibodies in CL patients. Results indicate 1) Using PCR-RFLP on the ribosomal internal transcribed spacer 1 region (ITS1), it was found that *L. major* is the main species causing CL in KSA. However, *L. tropica* is the only species found in the Southwest (Asir) and also in few cases from Al Madinah region. 2) Clinical presentations vary depending on the parasite species; ulcerated nodular lesions correlate with *L. tropica* infections and papular lesions are more frequent associated with *L. major* patients. 3) Interestingly, multiple lesions (up to 29 per patient) correlated only with *L. major* cases, whereas no more than 3 lesions were found in *L. tropica* patients. 4) Confirmed patients were referred for treatment with topical antifungals, followed by 1-2 courses of intralesional pentostam. Treatment response depends on several factors, including parasite species, clinical features, geographical location and the presence of secondary infections. Overall, ~82% of *L. major* cases responded to pentostam whereas 60% of *L. tropica* cases were resistant to the same drug. To our knowledge, this is the first epidemiological map of CL in the whole KSA.

779

PREVALENCE OF CHAGAS DISEASE IN BOLIVIAN IMMIGRANTS TO NORTHERN VIRGINIA

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American Trypanosomiasis (Chagas disease) is thought of as an illness confined to Central and South America by most U.S.-trained physicians. With massive immigration of citizens from Chagas-endemic countries to the U.S. over the past 3 decades, the likelihood of significant disease burden is high, but at present estimated rather than formally assessed. There is a large Bolivian population in Northern Virginia, whose locality of origin is a high-risk area for for Chagas disease. We performed a pilot project of screening for Chagas disease in this population. A screening station was established on 2 occasions at a mobile consulate for the Bolivian embassy. All interested adults were screened with a rapid test kit (Chembio), and follow-up testing sent to the CDC for confirmatory ELISA (Wiener assay) and IFA. All participants were from the Cochabamba region. Of 24 screened patients, 9(38%)were positive by Chembio assay. Confirmatory assays were positive in 9/9 patients. Screening for Chagas disease in a high-risk Bolivian population suggests the high likelihood of significant disease burden in the Washington DC area. A more rigorous screening program in this population is warranted.

780

CLINICAL CARDIAC FINDINGS DESCRIBING DISEASE SEVERITY IN *TRYPANOSOMA CRUZI* INFECTED PERSONS IN A BOLIVIAN URBAN PUBLIC HOSPITAL

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Trypanosoma cruzi, the parasite causing Chagas cardiomyopathy (CC), is the leading parasitic cause of morbidity and mortality in South America. Bolivia has the highest *T. cruzi* prevalence world-wide with infection rates of up to 43%. To evaluate ECG, echocardiogram, and serum-derived biomarkers of Chagas cardiomyopathy, we recruited 425 adults (48.7% male; mean age±SD, 57.5±12.7yrs) with and without *T. cruzi* infection from a large public hospital in Santa Cruz. We enrolled cardiac patients from the inpatient medicine service (n=128), cardiology clinic (n=94), and healthy controls (n=203). All patients underwent a cardiac history and demographic questionnaire for NYHA heart failure (HF) classification, blood draw, and ECG with QRS scoring. 250 patients also received an echocardiogram and 312 were chosen for serum biomarker analysis. Here we report clinical cardiac findings. By history, patients reported HTN (n=185, 43.4%), CAD (n=43, 10.1%), past pacemaker implantation (n=19, 4.5%), and HF symptoms (n=165, 38.8%). 324 patients (76.4%) were *T. cruzi* seropositive. For those in HF, no significant differences were noted in QRS score, EF, or left ventricular end diastolic diameter between seropositive and seronegative individuals. However, seropositive HF patients tended to have longer PR intervals (median 167 vs 153, IQR 153-192 vs 148-177), longer QRS duration (median 110 vs 102, IQR 97-149 vs 90-140), and lower heart rates (median 73 vs 86, IQR 65-92 vs. 69-94) than seronegative HF patients. All 9 cases of bradycardia in HF patients occurred in seropositive patients. Of seropositive individuals, characteristic Chagas EKG changes were more frequent in those with HF (n=134, 41.4%), with a significant difference ($p<0.001$) in the presence of atrial fibrillation, PVCs, right bundle branch block (RBBB), and left anterior fascicular block (LAFB). A hallmark Chagas finding, bifasicular block (RBBB with LAFB) was present in 13 seropositive individuals (4.2%). Forthcoming echocardiogram and serum biomarker results will provide a more in-depth evaluation of cardiac structural changes in this cohort and will help to determine reliable early indicators of CC risk. This would allow treatment to be targeted to patients with the highest likelihood of future morbidity and mortality.

781

SPATIAL ASSOCIATION BETWEEN *TRYPANOSOMA CRUZI*-INFECTED *TRIATOMA INFESTANS* AND *T. CRUZI*-SEROPOSITIVE DOGS IN AREQUIPA, PERU

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Chagas disease, a vector-borne disease transmitted by triatomine bugs and caused by the parasite *Trypanosoma cruzi,* affects 8 - 10 million people in the Americas. Indoor residual spraying campaigns are the most effective interventions to stop the transmission of the parasite and are routinely conducted in Arequipa, Peru to eliminate *Triatoma infestans*, the only vector in this area. Dogs are important reservoirs of *T. cruzi*; however, they have not been included as targets for interventions to halt the life cycle of the parasite. The objectives of this study were (i) to determine the seroprevalence of dogs in an area with re-emerging *T. infestans* following insecticide control; (ii) to describe the spatial distribution of dogs, *T. infestans* and *T. cruzi*; and (iii) to determine if proximity to infected triatomines is associated with higher risk of infection with *T. cruzi* in dogs. We conducted a cross-sectional serological screening to detect antibodies against *T. cruzi* in dogs, an entomological survey to collect vectors from households. We assessed spatial clustering of seropositive animals and infected vector colonies. Canine seroprevalence in the area was 13.0% (n=154). Infected colonies showed spatial clustering evaluated with K-function difference between 20 and 75 meters in constrast with seropositive dogs which did not show spatial clustering. The spatial intensity of all captured colonies and all sampled dogs evaluated with quartic kernels was homogeneous over the study area, but the spatial intensity of infected colonies and seropositive dogs showed zones with higher intensity and these areas overlap in most part. The adjusted odds ratio between seropositivity to *T. cruzi* and being close to an infected triatomine (defined as ≤50m) was 5.67 (95%CI: 1.12 – 28.74; p=0.036) after adjusting for age and sex of the dog. Interventions based on entomological data that are used to determine high-risk areas for humans could include the presence of dogs around houses where infected triatomines were collected to prevent future resurgence of the parasite.

782

MOLECULAR CHARACTERIZATION OF MILTEFOSINE UNRESPONSIVENESS IN *LEISHMANIA DONOVANI*

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Drug resistance is a major problem in leishmaniasis chemotherapy. Pentavalent antimonials have been the first-line drugs in the treatment of all forms of leishmaniasis for more than 70 years. But during last 20 years various cases of SbV resistance have been reported. There was need of some novel and effective antileishmanial drugs raised and thus miltefosine, paromomycin and amphotericin B used as replacement of SbV. During recent years these novel drugs also showed unresponsiveness in various parts of world. Understanding molecular mechanism behind the drug resistance is very crucial for success of such antileishmanial drugs. Genomic analysis has been performed primarily with SAG resistant *Leishmania* species and here we investigate molecular alterations in Miltefosine resistance in *L. donovani*. RNA expression profiling using microarrays is a suitable approach to study such events leading to a drug-resistance phenotype. We selected clinically Miltefosine unresponsive and responsive population of *L. donovani* promastigotes. Gene expression of both type of strains were studied using RNA microarrays. Genes having more than two fold of variation in expression used to validated by real-time RT-PCR. RNA expression profiling of Miltefosine unresponsive *L. donovani* revealed the over and down expression of few genes involved in drug resistance. Further study is going on for the validation of resistance molecular markers.

783

A PROTOCOL TO OPTIMIZE MICROSATELLITE DNA AMPLIFICATION OF *TRYPANOSOMA BRUCEI GAMBIENSE* FROM BODY FLUIDS

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Microsatellite genotyping of *Trypanosoma brucei gambiense*, the causative agent of human African trypanosomiasis or sleeping sickness, and population genetics tools, are useful for inferring population parameters such as population size and dispersal. Amplifying parasite DNA directly from body fluids (i.e. blood, lymph or cerebrospinal fluid) allows avoiding costly and tedious isolation phases. It is however associated to increased frequencies of amplification failures (allelic dropouts and/or null alleles). We present a study focused on improving microsatellite *loci* amplification of *T. brucei gambiense* from Guinean sleeping sickness foci. We checked for the real nature of blank and apparent homozygous genotypes of parasite DNA directly amplified from body fluids. We tested the effect of three different DNA quantities for different microsatellite *loci* of trypanosomes from different body fluids. Our results show that some initially blanks and homozygous genotypes happen to be actual heterozygous genotypes. In Guinea, lymph from the cervical nymph nodes, known to contain the highest concentrations of parasites, appeared to provide the best amplification results. Simply repeating the PCR may be enough to retrieve the correct genotype, but we also show that increasing initial DNA content provides better results while undertaking first amplification. We finally propose an optimal protocol for amplifying *T. brucei* DNA directly from body fluids that should be adapted to local characteristics and/or constraints.

784

ANTI-*LEISHMANIA DONOVANI* ANTIBODIES ENHANCE PROMASTIGOTES INTERNALIZATION INTO HOST MACROPHAGE

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Leishmania spp. promastigotes preferentially infect host macrophages, where parasite internalization is facilitated by several host and parasite surface molecules. This study aimed to demonstrate the role of humoral immunity in *Leishmania* parasite internalization into host macrophages. First, informed consent sera were obtained from 67 parasitologically confirmed visceral leishmaniasis patients reporting to our field treatment centre, Eastern Sudan. Then following titre determination, sera that had a titre of >102,400 were selected for parasite coating. An *in vitro* parasite internalization system was developed to enhance the *Leishmania*/macrophage interactions. The mean parasite number per monocytes was

626 ± 91 for antibody-coated *Leishmania donovani*, compared to 412 ± 70 uncoated isolates (p= 0.01). On the other hand, the percentage of infected cells was significantly higher for all antibody-coated isolates (100%) compared to uncoated ones (40%). This evidence of high infectivity probably points to the fact that anti-*Leishmania* antibodies facilitated the parasite uptake by host macrophages and monocytes-derived macrophages (MDM). Moreover, the rate of parasite uptake by MDM was significantly higher compared to monocytes (p= 0.00). This could be explained by the fact that the functional capabilities of fully differentiated macrophages differ from monocytes. In conclusion, host humoral immunity probably plays a pivotal role in *Leishmania* parasites internalization into host macrophages.

785

HIGHER PARASITE BURDEN IN HEALTHY (ASYMPTOMATIC) INDIVIDUALS CONTRIBUTE IN PROGRESSION OF VISCERAL LEISHMANIASIS

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In an area endemic for Visceral Leishmaniasis (VL), subclinical or asymptomatic infections play a crucial role in progression of disease. We determined the parasite load by quantitative PCR (qPCR) in healthy infected population living in an area endemic for Visceral Leishmaniasis. We enrolled 13366 persons from 11 villages of highly endemic region of Bihar, India. We conducted two sero- surveys with 1 year time interval for identification of incident infected healthy individuals. Parasite load using TaqMan based qPCR were done on these sero-converted individuals and its matched control populations. Individuals having parasite load greater than 1 genome/ml of blood was considered as positive by qPCR. Follow-up visit to the homes of each individual were made to monitor the disease conversion in this cohort. Agreements between seroconversion and qPCR were accessed by kappa value. Total 235 persons were converted their serology within 12 month intervals. Of these 235 sero-converters 105 (44.6%) individuals were also positive by qPCR. However, similar number of controls groups (87/ 237, 37%) also showed positivity by qPCR. The agreement between sero-converter and qPCR was moderate. Among all individuals only one were converted into disease that has parasite load 146 parasite genome/ ml of blood. These findings suggest the usefulness of parasite load in healthy individuals living in an endemic area of Bihar and contribute as a good tool for VL elimination programme.

786

PHENOTYPIC AND FUNCTIONAL EXAMINATION OF NEUTROPHILS FROM BRAZILIAN CUTANEOUS LEISHMANIASIS PATIENTS

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The protozoan parasite *Leishmania braziliensis* is the causative agent of the disease cutaneous leishmaniasis (CL) which can cause ulcerated skin lesions in individuals living in endemic regions. Skin pathology is thought to result from overabundant inflammatory myelocytic and lymphocytic cell responses. The effect of acute CL infection on neutrophil (PMN) mobilization and activation in the peripheral blood and how PMN might influence other cells of the immune response during disease remains unclear. In collaboration with investigators from the Federal University of Bahia (UFBA) in Salvador, state of Bahia, Brazil, we studied PMN from peripheral blood of patients with acute CL encountered in a rural leishmania-treatment center in Corte de Pedra, Bahia. We hypothesized that, as in other acute inflammatory models, peripheral blood PMN from CL patients would contain increased numbers of activated PMN with increased production of reactive oxygen species and the ability to suppress T lymphocyte responses. We detected PMN activation by higher CD66b and CD11b, and lower CD62L expression according to flow cytometry. We also detected a potentially suppressive, CD66b+ low-density PMN (LD-PMN) population in the PBMC fraction following centrifugation in a density gradient. Superoxide production was detected by spontaneous and PMA-induced ferricytochrome C reduction. T cell responses were detected by *in vitro* stimulation of peripheral blood mononuclear cells (PBMCs) with parasite antigen, followed by surface staining for Ki67 (proliferating cells) and intracellular staining for IFNγ when stimulated. Contrary to our hypothesis, we found similar levels of surface CD66b and CD11b and similar superoxide production in both CL patients and controls, but decreased CD62L in CL patients, indicating a difference in some, but not all measures of PMN activation. We detected a population of potentially suppressive CD66b+ LD-PMN in the PBMC fraction of CL patients. However, co-incubation of PMN of normal density with PBMCs resulted in enhanced, rather than suppressed, T cell responses. Surprisingly, a population of PMN with increased expression of the MHCII complex HLA-DR was observed in CL patients. We conclude that human peripheral blood PMN are not suppressors of T cell responses in subjects with CL. Ongoing studies will determine whether the unique PMN subset expressing HLA-DR is capable of presenting antigen to T cells during human CL.

787

PARASITOLOGICAL CONFIRMATION OF ASYMPTOMATIC INFECTION IN ENDEMIC AREAS FOR CUTANEOUS LEISHMANIASIS IN COLOMBIA

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Asymptomatically infected individuals in endemic areas for cutaneous leishmaniasis (CL) can be a hidden and large reservoir of parasites available for anthropophilic vectors. Viable *Leishmania* persist indefinitely after clinical resolution of disease. Whether parasites persist during asymptomatic infection and its impact on disease transmission is unknown. We aimed to parasitologically confirm asymptomatic infection and the relative parasite burden in mucosal tissues and peripheral blood of inhabitants of communities endemic for CL in Colombia. Individuals from 4 communities in Nariño and Risaralda were included. Participants were categorized as uninfected, asymptomatically infected; healed CL or active CL. Blood monocytes, lesion and scar aspirates, and nasal mucosa, conjunctiva and tonsil swabs were obtained from participants. Detection of L. Viannia kDNA was performed by PCR, and parasite viability confirmed by 7SLRNA RT-qPCR In communities of Risaralda the population was predominantly represented by individuals with history of CL (67%). kDNA positivity was found in 43% of individuals. Samples with the highest frequency of kDNA positivity were blood monocytes (37%) and nasal swabs (15%). Communities in Nariño were largely composed of uninfected individuals (63%) followed by those with history of CL (25%) and asymptomatic infection (9%), of which 27% were kDNA positive; swabs of tonsil (24%) and nasal mucosa (15%) were the most frequently positive samples. Samples with highest parasite burden were tonsil and nasal mucosal swabs Parasite persistence in individuals without clinical signs of infection highlights their potential in transmission. High frequency of kDNA positive blood samples in individuals with history of CL supports their previously unrecognized role as a source of parasite acquisition by the vector. Parasite detection in mucosal tissues may identify potential risk of disease activation. Understanding of parasite persistence in asymptomatic infection and healed CL in transmission is needed for the development of community-targeted control strategies.

788

GLOBAL METHYLATION CHANGES IN THE HEART DURING THE ACUTE INFECTION IN A CHAGAS RAT MODEL

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is the most costly parasitic disease of the Americas in terms of morbidity and mortality. In one third of infected patients, it produces severe and progressive injury of the heart, nearly always associated with a fatal outcome. The mechanisms of interaction, invasion, and pathogenesis in this disease are not only extremely complex, but also not fully understood. Genome methylation, a subarea in the study of epigenetics, has recently showed relevant information about distinct pathogenic scenarios, showing modulations as a consequence of inflammation, cancer, infection, fibrosis, among others. In this study we aimed to detect cardiomyocyte genome methylation levels modulations as a consequence of the heart injury during the acute stage of infection of T.cruzi in a rat model. 8 Holtzman-strain, 6-8 week old male rats were peritoneally inoculated with 107 metacyclic trypomastigotes, "Arequipa" strain, and sacrificed at 36 days post infection. 8 rats of the same strain were not infected and served as controls. Active infection was interrogated by microhematocrit method, PCR, and anti-IgG TESA-ELISA. The heart was removed immediately after death and fixed in formalin 3.7% formaldehyde in PBS. Methylation status was examined by semiquantitative, immunohistochemical evaluation of whole heart sections using a monoclonal antibody against 5-methylcytosine, considering that a direct relationship exists between genome methylation levels and intensity of the nuclei staining. At least 100 cardiomyocyte nuclei were blindly examined. All (8/8) rats presented a decrease in the levels of cardiomyocyte nuclei intensity, compared to controls ($P<0.05$). All methods of diagnosis confirmed the infection in all inoculated subjects. Our results indicate that as a consequence of the acute infection of *T. cruzi*, a significant decrease of genome methylation levels occurs in affected heart myocytes *in vivo*.

789

A CONGENITAL RAT MODEL OF CHAGAS DISEASE

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Trypanosoma cruzi is responsible for Chagas Disease. Usually, the infection occurs in the childhood, and most cases are evident in the chronic phase, when irreversible cardiac damage is diagnosed, may inexorably lead to death. To the present date there are unanswered questions about the mechanism of *T. cruzi* to cross placental barrier and infect the fetus. Additionally, there is a lack of a good diagnostic test in order to start early the treatment of the positive newborns to congenital Chagas, which is of vital importance, because benznidazole and nifurtimox are effective only in the acute phase of the disease. Our aim was develop an animal model for congenital Chagas which could help to the research. Six female Holtzman rats were inoculated by intraperitoneal route with 10^7 blood trypomastigotes from Arequipa isolate; control group was inoculated with saline. Three days after the start of the mating period, females were inoculated. The observation of a vaginal plug determined the pregnancy day one. Parasitemia was evaluated at 10 and 19 days post inoculation (dpi). Necropsy was performed over 19 dpi, blood samples of the pups were evaluated by microconcentration technique, serology and molecular test. Placenta and umbilical cord were collected and immersed in absolute ethanol; also, placenta was collected in phosphate buffer solution pH 7,2 for histopathological analyses. Parasitaemia of the mothers peaked at 10dpi with a media of log 3.4 parasites/ml, while at 19dpi it decreased to a media of log 3.4 parasites/ml. Of 44 neonates analyzed so far, which were born to an infected mother, 70.45% (31 of 44) were positive for the Arequipa strain using PCR/qPCR. Random samples of this Arequipa group were subject to further analysis by histology; amastigote nests were found in 28.57% (4 of 14) respective placentas. Similarly, 33.33% (7 of 21) neonate blood clot samples were positive for anti-TESA IgM antibodies by ELISA, and 4.55% (2 of 44) neonates exhibited *parasitemia* in their amniotic fluid. Our experimental model of congenital Chagas transmission allowed us to obtain infected as well as no infected pups samples from the same mother, which is a great advance to perform studies to improve the know ledge about routes of transmissionand allowing the evaluation of diagnosis techniques as an alternative to the human studies and the troubles and costs of what that implies.

790

MODULATION OF ACTIVATION-ASSOCIATED MICRORNA ACCUMULATION DURING MONOCYTE-TO-MACROPHAGE DIFFERENTIATION

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Circulating monocytes recruited to tissues can become monocyte-derived macrophages (MDMs). Monocytic cell gene expression programs are influenced by environmental cues such as cytokines and cell-cell interactions. The regulation of microRNA (miRNA) expression in response to these environmental cues is not as well characterized. We recently reported high basal expression of several activation-associated miRNAs in primary human MDMs relative to freshly isolated peripheral monocytes. This observation suggests that accumulation of key miRNAs occurs as part of monocyte-to-macrophage development. We hypothesized that the rate of activation-associated miRNA accumulation could be modified in monocytic cells responding to environmental stimuli during differentiation. Indeed, the rate of miR-193b and miR-222 accumulation was augmented by IL-4 treatment. While LPS stimulation augmented miR-146a and miR-155 expression, the accumulation miR-125a-5p and miR-222 was antagonized by IFN-beta or IFN-gamma. Toward determining a mechanism underlying activation-associated miRNA accumulation, we found that expression of primary miRNA, not Dicer, directly correlated with activation-associated miRNA expression. Interestingly, miR-155 and miR-193b accumulation was greater in MDMs differentiated within total PBMC cultures than as purified monocyte cultures. Both of these miRNAs were also highly expressed in purified monocytes co-cultured with non-monocytic PBMCs. In summary, the rate of activation-associated miRNAs accumulation during monocyte-to-macrophage differentiation can be modified not only through defined stimuli such as LPS and cytokine treatments but also through an uncharacterized interaction between monocytic and nonmonocytic cells. Biologically-important miRNAs in macrophage differentiation and activation will be assessed for the capacity to polarize macrophages towards a M1 phenotype that induces microbicidal responses toward intracellular pathogens such as *Leishmania*.

791

INFECTIVITY STUDY OF THREE RODENTS HOSTS OF *LEISHMANIA (VIANNIA) BRAZILIENSIS*: POSITIVITY, PARASITE LOAD AND TISSUE TROPISM

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For over three decades, the primary reservoirs of *Leishmania (Viannia) braziliensis* were not known, but the development of PCR protocols to detect parasites in samples obtained from sylvatic animals implicated some vertebrate species such as rodents and marsupials as primary reservoirs of *L. (V.) braziliensis*. To observe the infection profile in some of these reservoirs, animals from established colonies of *Nectomys squamipes*, *Necromys lasiurus* and *Rattus rattus* were experimentally infected with 10^6 promastigote forms of *L. (V.) braziliensis*. After six weeks of infection, samples of skin, spleen and liver were processed to purify template DNA for detection and quantification PCR assays, using DNeasy Blood and Tissue kit (Qiagen), according to manufacturer's protocol. The initial detection protocol consisted in a nested PCR assay using two pairs of SSU rDNA (Small Subunit Ribosomal gene) derived oligonucleotides. The first PCR used primers that amplify a conserved region in all trypanosomatids and the second reaction used primers that amplify a common region of the *Leishmania* genus. The quantification protocol consisted in a real time SYBR-Green PCR, wherein the parasite load was estimated by normalizing the number of SSU rDNA copies per host glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) copy number. We observed a higher positivity in samples of *N. lasiurus* and *N. squamipes* indicating that these species are more susceptible than *R. rattus*. However, all samples presented a low parasite load, 11.4, 128 and 235 SSU rDNA copies in *R. rattus, N. squamipes* and *N. lasiurus* samples, respectively, what correspond to the DNA of at most one parasite/50 ng of host DNA. Besides, we were not able to observe parasite tissue tropism for the three analysed species. The high positivity in samples of *N. lasiurus* and *N. squamipes*, and concomitant low parasite load, may be features that support the life cycle of the parasite, ensuring the role of these rodents as reservoirs in sylvatic cycle.

792

IS OVER-DEPENDENCE ON MALARIA RDTS DISGUISING MORBIDITY DUE TO OTHER DEADLY HEMOPARASITES AT PERIPHERAL HEALTH FACILITIES?

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Microscopy and multispecies malaria RDTs are standard laboratory tests for febrile patients suspected of having hemoparasites at district health centers and rural health posts, respectively, in Ethiopia. Significant number of malaria and relapsing fever (*Borrelia recurrentis*) cases are reported among seasonal migrant workers during major malaria transmission season in endemic areas. A cross-sectional study was conducted to compare the laboratory diagnosis and treatment of outpatient febrile patients at 2 district health centers and 11 satellite rural health posts during the major malaria transmission season in central Ethiopia. Demographic, clinical and laboratory data were abstracted from the monthly morbidity reports of each facility. Laboratory tests were done for 2326 and 711 patients in district health centers and health posts, respectively. 170 (5.6%) of all total febrile patients were migrant seasonal workers of which 106 (62.4%) were seen at the health centers. Prevalence of malaria at health posts was 13.9% (99/711): 53 *P. falciparum* infection, 44 non-*falciparum* malaria and 2 mixed infections. Prevalence of malaria at health centers was 9.3% (217/2326) of which 97 (44.7%) *Plasmodium falciparum*, 112 (51.6%) *P. vivax*, and 8 (3.7%) mixed infections. The overall prevalence of *B. recurrentis* infection was 1.4% (33/2326), of which 12 were migrant workers making the subgroup prevalence 11.3% (12/106). In conclusion, detection and species identification of plasmodium infections by both microscopy and multispecies RDTs tests, and relapsing fever by microscopy guided rational drug administration at peripheral health care facilities. *B. recurrentis* is an important etiologic agent of febrile illness in the region. However, non-reporting of relapsing fever at the health posts can be attributed to diagnostic limitation with risk of missed diagnosis of the highly contagious and potential life-threatening pathogen. Diagnosis and treatment algorithms for febrile patients in endemic areas of multiple etiologies such as plasmodia and Borellia should take in to consideration epidemiology and seasonality. More systematic study involving microscopy-RDT parallel testing and monitoring of treatment outcomes during major transmission seasons might shade more light on the extent of the problem.

793

Γ-RAY POLYMERIZED NANOSPHERES FOR SEROLOGICAL DIAGNOSIS OF MALARIA

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Malaria is a major public health problem especially in tropical and sub-tropical regions of the world. The methods most commonly used to diagnose malaria patients sera, such as IFAT and ELISA, are not easy methods to examine in the bedside and the clinical laboratory in a hospital. Here, we wish to report the development of novel peptide immobilized polymer nanospheres for the detection of specific antibodies in malaria patients sera. The peptide antigens (~20 residues) were designed from two *Plasmodium* specific sequences in lactate dehydrogenase (pLDH) and in enolase (AD22). Each test material is prepared by chemical coupling of peptides to the surface of the diethylene glycol dimethacrylate and methacryloyl-OSu copolymer nanosphere. At the beginning of agglutination test, serum samples were 2-fold serially diluted (1/16···1/16384) with phosphate buffered saline in simple 96-well microplates with U-shaped bottom. Then, the nanospheres suspension was added to the microplates, which were followed by vigorous agitation. In the microplates, aggregation patterns can be observed almost at 4-6 h after the reaction, when left at room temperature. We have compared the reactivity against malaria patients' sera collected in endemic regions of the Philippines: (a) Mixed infected-, (b) *falciparum*-, (c) *vivax*-malaria patients, and (d) feverish patients (malaria negative). Successful results were observed for the pLDH material, which showed good specificity for three kinds of malaria patients compared with feverish patients. Interestingly, in the case of AD22 material, malaria patients did not show distinct titer values from feverish patients. This is due to the difference of antibody persistence against enolase (AD22) and lactate dehydrogenase (pLDH) in the endemic area where residents are sequentially infected by *Plasmodium* parasites. Therefore, these results probably indicates that pLDH and AD22 antigens are suitable to detect present and recent-past infections, respectively.

794

EVALUATION OF THE MALARIA RAPID DIAGNOSTIC TEST VIKIA MALARIA AG PF/PAN™ IN ENDEMIC AND NON-ENDEMIC SETTINGS

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Malaria rapid diagnostic tests (RDTs) are a useful tool in endemic malaria countries, where light microscopy is not feasible. In non-endemic countries they can be used as complementary tests to provide timely results in case of microscopy inexperience. This study aims to compare the new VIKIA Malaria Ag Pf/Pan™ RDT with PCR-corrected microscopy results and the commonly used CareStart™ RDT to diagnose *falciparum* and non-*falciparum* malaria in the endemic setting of Bamako, Mali and the non-epidemic setting of Lyon, France. Blood samples were collected during a 12-months and six-months period in 2011 from patients suspected to have malaria in Lyon and Bamako respectively. Discordant results were corrected by real-time PCR. Samples of 877 patients from both sites were included. The VIKIA Malaria Ag Pf/Pan™ had a sensitivity of 98% and 96% for *Plasmodium falciparum* in Lyon and Bamako respectively, performing similar to PCR-corrected microscopy. The VIKIA Malaria Ag Pf/Pan™ performs similar to PCR-corrected microscopy for the detection of *P. falciparum*, making it a valuable tool in malaria endemic and non-endemic regions.

795

PREVALENCE OF ASYMPTOMATIC MALARIA INFECTIONS IN COLOMBIAN REGIONS WITH DIFFERENT EPIDEMIOLOGICAL PROFILES

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This study evaluated the prevalence of *Plasmodium falciparum* and *P. vivax* asymptomatic infections in three different malaria regions of Colombia with different epidemiological profiles: Tierralta (Ta), Tumaco (Tu) and Buenaventura (Bv). We conducted cross sectional surveys in 8 sentinel sites in which 1,170 subjects from 267 households were studied. Participants were asked to respond to a Knowledge, Attitudes and Practices (KAP) questionnaire and then were bled to determine the prevalence of malaria infection and sero-prevalence. Malaria diagnose was carried out using blood thick smears (TS) and RT-qPCR, whereas serology was assessed by IFAT and ELISA. The KAP survey indicated that besides high knowledge scores in the three communities, there are gaps in practices such as treatment adherence and use personal protection measures. In addition, we found negative correlation between socio-demographic scores and the seropositivity (PvMSP-1) with P value of 0.025. Whereas the overall prevalence of asymptomatic infection measured by TS was ~1%, by RT-qPCR it was 6.4% (n=45), with a greater proportion (21%) in 40-50 years old individuals. Furthermore different regions displayed different prevalence of asymptomatic infections: Bv 12%, Ta 1% Tu 4.3%. From these 45 samples, 66.2% were positive for *P. vivax* and 33,8% for *P. falciparum*, which correlates to the overall parasite prevalence in Colombia. While IFAT serology indicated greater recognition of *P. falciparum* (53%) than of *P. vivax* (18%), ELISA analyses indicated that 63% percent of reactivity to *P. vivax* (40%= MSP-1 and 39% PvCS) with 17% of double positives correlating with previous malaria episode of the population (~50%). We found highest reactivity index (51%) in Tu, concordant with the high malaria incidence, followed by Ta (49%) and Bv (19%). This study strengthen the importance in conducting active case surveillance as mean to more accurately determine malaria incidence, as well as to eliminate malaria reservoirs, and the need to use technique more sensitive than TS.

796

WILL CAREGIVERS OF UNDER-FIVE CHILDREN IN RURAL GHANA ACCEPT TEST-BASED MANAGEMENT OF MALARIA?

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The shift to test-based management of malaria (TBMM) is a departure from the presumptive approach that caregivers of under-five children in high transmission settings have been used to for many years. We used a survey with logistic regression analysis to explore the determinants of the willingness of caregivers in rural Ghana to accept TBMM. We followed it up with focus group discussions to explore the major emergent issues. A total of 3047 caregivers were interviewed. Nearly all (98%) reported a preference for TBMM over presumptive treatment. Caregivers who preferred TBMM were less likely to be concerned about the denial of ACT to their test-negative children (O.R. 0.57, 95% C.I. 0.33 -0.98). Caregivers who had valid (adjusted O.R. 1.30, 95% CI 1.07-1.61) or expired (adjusted O.R. 1.38, 95% CI 1.12-1.73) insurance cover were more likely to be concerned about the denial of ACT to their RDT-negative children than caregivers who had never secured national health insurance cover. Perception that a blood test at health centre level represents improvement in the quality of care, leads to improvement in treatment outcomes, and offers opportunity for increased communication between health workers and caregivers are factors that promote acceptability. Acceptability is also enhanced by engaging caregivers in the procedures of the test. Apprehensions about negative health worker attitude could however undermine acceptance. The high acceptability of test-based management of malaria among caregivers in this population is because of the expectation that it will lead to improvement in the quality of care.

797

MALARIA CASE MANAGEMENT IN PAPUA NEW GUINEA PRE- AND POST-IMPLEMENTATION OF A REVISED TREATMENT PROTOCOL

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This study aimed to document malaria case management practices in Papua New Guinea pre- and post-implementation of a revised national malaria treatment protocol. The revised protocol stipulates routine testing of malaria infection by rapid diagnostic test (RDT) or microscopy, anti-malarial prescription to test positive cases only, and the introduction of artemether-lumefantrine (AL) as the first-line anti-malarial. The study hypothesized that the availability of malaria RDTs and AL and their use would increase over time, whilst the overall number of anti-malarial prescriptions would decrease. Data were collected via three countrywide cross-sectional surveys of randomly selected health facilities in 2010 (pre-implementation), 2011 (during implementation) and 2012 (12 months post implementation). Collectively, a total of 255 health facilities were surveyed across this time and 1568 malaria case management patients were observed. All data were collected using structured survey instruments. Only preliminary analyses were completed at the time of drafting this abstract. These data indicate a substantial increase in the

availability of malaria RDT or microscopy between 2010 and 2012 (15.2% and 53.4%, respectively) and a similar increase in the availability of AL (0%, 51.5%, respectively). In 2010, only 18.6% of observed febrile patients had a malaria RDT or blood slide taken, although 96.4% were prescribed an anti-malarial. Comparative data from 2012 have yet to be analysed. It is anticipated that the resulting findings, due to be completed in June 2013, will provide a clear indication as to the availability of diagnostic and treatment resources required for implementation of the new protocol as well as the level of health worker adherence to it.

798

IMPROVING QUALITY CONTROL OF THE MICROSCOPIC DIAGNOSIS OF MALARIA IN SENEGAL, 2009-2011

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Blood film microscopy is considered the gold standard for diagnosis of malaria, but is dependent on the technical ability of the slide reader. High quality malaria microscopy has been difficult to implement outside reference laboratories. In order to improve quality of malaria microscopy in hospitals and health centers, the Senegal National Malaria Control Program (NMCP) adopted a strategy of giving district and select hospital laboratory technicians a week of intensive training followed by supervision visits during which slides were taken from each laboratory for quality control. In 2009, 42 technicians were trained, with average pre-test and post-test scores of 55% and 64%, respectively. With expert microscopist reading as the gold standard, of 596 slides selected for quality control, trainees had a sensitivity of 95% and specificity of 73%, with 6.5% of slides unreadable. In 2011, 53 technicians were trained, with average pre-test and post test scores of 75% and 87%, respectively. Among 866 slides selected for quality control, sensitivity was 95% and specificity was 77%, with 1.8% of slides unreadable. While laboratory technicians read slides with a high degree of sensitivity and reasonable specificity, the quality of slide preparation and coloration was noted to be mediocre, and very few technicians performed speciation or parasite density. To further improve quality of microscopy at hospitals and health centers, in 2013 regional biologists will be trained to help with supervision, the technician from each of the 76 district health centers will be retrained, all hospital and district health center labs will receive onsite supervision including proficiency testing from a standardized panel, and slide quality control. The microscopy quality control of the Senegal NMCP follows the recommendations of the World Health Organization in health facilities nationwide for parasite detection, speciation, and quantification of *parasitemia*. Improving quality of blood film microscopy at the peripheral level is important to improving diagnosis of low level *parasitemia* in the setting of decreasing transmission.

799

CORRELATING QUANTITATIVE REAL-TIME PCR TO RAPID ANTIGEN DETECTION ASSAY AND QUALITATIVE PCR RESULTS IN ISOLATED *PLASMODIUM FALCIPARUM GAMETOCYTEMIA*

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Microscopic examination of stained thick and thin blood smears is the gold standard method for laboratory diagnosis of malaria, and can differentiate clinically relevant asexual *parasitemia* from clinically irrelevant isolated *gametocytemia*. Microscopy is time consuming, labour intensive, and requires significant technical expertise to perform. Rapid antigen detection assays are less labour intensive, but still may not reliably differentiate isolated *gametocytemia* from asexual *parasitemia*, which is important in a non-endemic laboratory context. To determine if Ct values on *Plasmodium* genus and *P. falciparum*-specific PCR assays targeting the 18S rRNA gene correlate to positivity of rapid antigen detection assay (Binax NOW Malaria), and 18S rRNA gene copy number, we analyzed samples from Ontario patients with isolated *P. falciparum gametocytemia*. Thirty-one samples containing microscopically-confirmed and isolated *P. falciparum* gametocytes, and no asexual stages, were identified and analyzed. Of these, 23 (74%) were follow-up specimens from patients known to have prior samples positive for asexual stages of *P. falciparum*, and 3 (10%) were from patients previously known to have mixed asexual stages of *P. falciparum* and *P. vivax*. Twenty-nine of 31 (93.5%) samples with isolated *gametocytemia* were positive for *Plasmodium falciparum*-specific histidine rich protein-2 (HRP-2) by rapid antigen detection assay. Nine of 31 samples (29.0%) were positive for *Plasmodium* genus aldolase by rapid antigen detection assay. Positivity of the aldolase band on rapid antigen detection assay was significantly correlated to lower mean *Plasmodium* genus ($p=0.002$) and *P. falciparum*-specific Ct values ($p<0.001$) by qualitative real time PCR. In addition, positivity of the aldolase band on rapid antigen detection assay was significantly correlated to higher mean *P. falciparum* 18S rRNA gene copy by quantitative real time PCR ($p=0.001$). These findings underscore that rapid antigen detection assays and conventional PCR assays do not reliably distinguish sexual from asexual *parasitemia* in a laboratory setting where clinical information may be unavailable. In addition, that rapid antigen detection assays can detect isolated *gametocytemia* has public health relevance in malaria endemic areas. Simple and rapid tests that can differentiate asexual from isolated sexual *parasitemia* are needed.

800

THE APPLICATIONS OF TRANSCRANIAL DOPPLER FOR THE ASSESSMENT OF INCREASED BRAIN VOLUME IN RETINOPATHY POSITIVE CHILDREN WITH CEREBRAL MALARIA

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Having established a strong association between death and markedly increased brain volume (BV) per magnetic resonance imaging (MRI) in children with cerebral malaria (CM), we are now evaluating potential etiologies and surrogate measures of increased BV. Seven children with retinopathy-positive CM were evaluated with serial MRI studies and serial Transcanial Doppler (TCD) measurements. Patients were categorized on the basis of BV estimates (per MRI), and three TCD-derived values: mean cerebral blood flow ve*loci*ty (mCBFV), pulsatility index (PI, [systolic -diastolic]/mean ve*loci*ty) and Lindegaard ratio (mCBFV MCA/mCBFV ICA) into three categories: Increased BV with raised intracranial pressure, hyperemia, and "Hemodynamically Stable". The patients with increased BV and raised intracranial pressure were characterized by a clinically significant decrease in mCBFV coupled with an increase in PI which was accompanied by an increase in BV per MRI. In contrast, the hyperemia group showed an increased mCBFV and relatively low Lindegaard ratio during increased BV per MRI. Those deemed 'hemodynamically stable' remained unchanged for both the TCD and MRI measurements. Our findings demonstrate that repeated TCDs may provide information about BV, as confirmed by MRI, and may illuminate potential etiologies for increased BV in African children with CM. The point-of-care monitoring of TCD would allow clinicians to modify treatments at the bedside without the need of an MRI.

801

EXTENT AND DETERMINANTS OF MALARIA DIAGNOSTIC TEST USE AMONG FEBRILE CHILDREN UNDER FIVE IN THIRTEEN SUB-SAHARAN AFRICAN COUNTRIES USING STANDARDIZED NATIONAL SURVEYS

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In 2010, the World Health Organization revised its malaria treatment guidelines to recommend diagnostic testing of suspected malaria cases before starting treatment. New data on malaria testing are available from national population-based surveys to examine the extent and determinants of malaria diagnostic test use among febrile children under five at the outset of this recommendation. We reviewed DHS, MICS and MIS in sub-Saharan Africa for inclusion of the main outcome: caregivers' reports that a child had fever in the previous 2 weeks and a finger/heel stick for testing. Surveys also needed to report main predictors: care sought for fever by facility type/level and malaria transmission intensity linked to datasets through geocoded survey clusters. 13 datasets met these inclusion criteria. We estimated total febrile children under five tested in 2010 in studied countries based on previously published methods. We examined factors associated with test uptake across pooled datasets using a multilevel logistic regression model with individuals nested within clusters and country as a random coefficient. Odds ratios for main predictors were adjusted for socioeconomic factors. Preliminary results indicate 4,896 (17%) febrile children under five were tested across studied countries. Compared to hospitals, the odds of a febrile child getting tested decreased by 37% if attending a non-hospital facility and by 68% if visiting a community health worker. Compared to no malaria risk settings, febrile children in low or moderate stable transmission areas were twice as likely to be tested. Febrile children with poorly educated mothers or living in poorest households were tested significantly less often than counterparts. Future analyses will examine differences in test uptake associated with attending public/private facilities as well as stratification of results for rural and urban contexts. Findings to date suggest significant inequities in malaria testing. An important step to close this gap is to prioritize test roll out to lower levels of care. This could improve fever management quality and more rational drug use where most non-complicated pediatric fevers are managed, particularly among poorest children.

802

IS THIS EVIDENCE OF SUCCESS IN MALARIA PREVENTION AND CONTROL MEASURES? IMPACT OF PREVENTION AND CONTROL MEASURES IN NIGERIA

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Malaria is a preventable and treatable infectious disease which is a major public health issue in sub-Saharan Africa. Nigeria bears up to 25% of the malarial disease burden in Africa, hence contributing significantly to the one million lives lost per year in the region. The financial loss due to malaria is estimated to be about 132 billion Naira (8.8million US dollars). Efforts by the Federal Government and other stakeholders in the areas of mass distribution of insecticide treated nets (ITNs), community awareness programs, promotion of Artemisinin-based combination therapies (ACTs) and biolarviciding in order to achieve the sixth Millennium Development Goal of combating malaria have contributed to reduction in malaria incidence from 60 % to 43 % in Rivers State, Nigeria. There is need to provide evidence-based data to extrapolate the effect of these interventions on the overall burden of malaria in Nigeria. This study investigates malaria prevalence amongst asymptomatic subjects presenting for routine medical examination at the Lulu Briggs Health Centre at the University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. Finger-prick blood samples were collected from 354 subjects, and were tested for parasitaemia using standard Rapid Diagnostic Test Kits (RDT; SD and Abbon), genotype, blood group and packed cell volume. Of the 354 samples tested, 39 (11%) tested positive for *Plasmodium* parasite. Among these, 32 (82.1%) were of AA, 6 (15.4%) AS, and 1 (2.5%) SSgenotype. Of the 39 samples positive for *Plasmodium* parasite, 23 (59%) were of O+, 10 (25%) of A+, 3 (8%) of AB+, and 3(8%) of the B+ blood groups. These results show an 11% prevalence of malaria among subjects studied. The results suggest that malaria control and prevention measures are having a degree of success in the country. This success may be the result of the aggressive campaigns to scale up malaria control measures, especially, prevention tools, like ITNs. These gains are impressive but must be sustained to avert a possible resurgence in the wake of ACT-resistance.

803

MICROENCAPSULATION INCREASES THE ANTIMALARIAL EFFICACY OF *PAVETTA CRASSIPES*

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The aim of this work was to develop *Pavetta* (PV)-loaded microcapsules and evaluate its antimalarial efficacy *in vitro*. Microcapsule dosage form with sodium alginate containing *Pavetta crassipes* alkaloidal extract was prepared using ionotropic gelation technology. The efficacy of the *pavetta*-loaded microcapsules was evaluated in chloroquine-sensitive (HB3) and chloroquine-resistant (FCM29) *Plasmodium falciparum* strains. Three standard drugs; artemisinin, chloroquine and quinine were used as reference standards for comparism. *Pavetta*-loaded microcapsules presented an adequate particle size (173 nm), narrow particle distribution (0.20), positive zeta potential (14mV) and high drug content (98.88 %) and encapsulation efficiency (67.01 %). The extract exhibited significant ($P < 0.05$) antiplasmodial effect against both *Pf* HB3 and *Pf* FcM29. The efficacy of *pavetta*-loaded microcapsules as measured by *in vitro* assay doubled (11.20) when the extract was microencapsulated compared with the free extract (22.08). IC_{50} of 11.20 and 10.60 µg/ml for *Pf* HB3 and *Pf* FcM29 respectively, representing an almost 50 % increased activity were recorded for *Pf* HB3 and *Pf* FcM29 respectively, compared with the IC_{50} of 22.08 and 21.91 µg/ml respectively for the free extract. Therefore, microencapsulation increased the interaction between the extract and the erythrocyte and this mechanism may be responsible for the extract's increased efficacy when microencapsulated. Our current findings show that PV and its microcapsule formulation may be a useful preparation in the treatment of acute chloroquine-sensitive and chloroquine resistant malaria.

804

ANTIPARASITIC ACTIVITY OF LACTOFERRIN PROTEIN ON *PLASMODIUM FALCIPARUM* AND LACTOFERRIN BOUND TO CHITOSAN NANOPARTICLES ON *P. BERGHEI* INFECTED MICE: AN *IN VITRO* AND *IN VIVO* STUDY

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Lactoferrin protein is known as antimicrobial, antiparasitic, antitumor and immunomodulating in nature. The protein has shown its effect against

many parasites like *Giardia, Entamoeba.* But until now there is no study which has shown its inhibitory effect on malarial parasites *in vitro* as well as *in vivo*. Different concentrations of apo and Fe saturated lactoferrin at 40 and 80ug/ml were incubated with infected RBC's at initial *parasitemia* of 1%. After 24 and 48 hrs, the giemsa smear of the treated and non treated group were made and observed microscopically. ROS production and the *parasitemia* mean in all above groups were observed by flow cytometry (FACS). Also presence of ring, trophozoite and schizont was observed by confocal microscopy in treated as well as untreated groups. Study was divided in 2 groups of Balb/c mice labelled as: Infected mice with lactoferrin loaded Nanoparticle diet (given orally), infected mice fed on normal diet. Mice were inoculated with $1x10^6$ parasitized RBC's through I/P route. Following parameters were studied in all infected and uninfected groups at day 3, 6, 9 ,12 post infection: *parasitemia* in tail vein blood, spleen and liver weight and survival rate. The *parasitemia* count and ROS prodution was found to be decresed was found to be significantly decresed in Fe saturated lactoferrin protein group and apo protein group at 40 and 80 mg/ml concentration at 24 and 48 hrs post infection when compared with untreated group ($p<.005$). The malaria disease was developed in only 40% of the infected mice. At day 9 the *parasitemia* in case of normal diet mice was found to be >50% as compared to nanoparticles diet mice which showed only 10-15% *parasitemia* ($p<.005$). While mice with normal diet started dying on day 9 post infection. The mice given these nanoparticles orally resulted in survival upto 25 days. Two mice died at day 9 in case of normal diet group. The mice having normal diet showed enlargement of spleen and liver due to parasite sequestration as compared to mice which were on nanoparticles diet. ($p<-005$). In conclusion, the present study has shown good inhibitory effect of lactoferrin protein on intracellular parasites. Lactoferrin can be used as a theraputic drug for malarial infection.

805

THE *IN VIVO* ANTI-PLASMODIAL ACTIVITY OF *NUCLEALA LIFOLIA* ELIMINATES CEREBRAL AND HEPATIC PARASITEMIA AND REDUCES DAMAGE TO THE TISSUES' MICROSTRUCTURES

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The high rate of malaria transmission and prevalence have been reported in the Niger-Delta wetland region of Nigeria. The inhabitants of this region rely heavily on local medicinal plants to treat malarial infection. One of such plant is *Nuclea latifolia* known to contain monoterpeneindol alkaloids. In this study, the *in vivo* antiplasmodial activity of *N. latifolia* and its potency to eliminate hepatic and cerebral malarial parasites, and abate oxidative tissue damage were investigated using mice model. Results show that the two doses (200 and 300mg/kg) of*N. latifolia*leaf extract significantly suppressed blood parasitaemia by 63.32% and 81.39%, respectively. These values compare well with the standard choloquine treatments whose chemosuppression was 90.39%. The leaf extract and chloroquine treatments eliminated hepatic and cerebral parasites, and abated the *Plasmodium berghei*-induced oxidative damage as indicated by the histopathological microstructures. *N. latifolia* holds promise in the treatment of malaria. Therefore, the bioactive ingredient should be structured and its mechanism of action deciphered in order to further understand the antiplasmodial activity of *N. latifolia.*

806

CARDIAC SAFETY OF MONTHLY DIHYDROARTEMISININ-PIPERAQUINE FOR MALARIA PROPHYLAXIS: A TWO ARM, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED COHORT STUDY

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Dihydroartemisinin-piperaquine (DP), the current first line recommended treatment of uncomplicated *Plasmodium falciparum* and *P. vivax* in Cambodia, and worldwide has previously been shown to be of benefit as a monthly medication to prevent malaria. We evaluated the safety and efficacy of monthly DHA-piperaquine in a compressed 2 day dosing regimen. Protective efficacy and cardiac safety of a monthly course of Dihydroartemisinin-piperaquine were compared in a two arm, randomized, double-blind, placebo-controlled cohort study with 2:1 treatment allocation. Healthy volunteers in high risk areas along the Thai-Cambodian border were administered 180 mg DHA and 1440 mg PIP as combination tablets for 2 days once per month within 1-3 hours of a meal. Volunteers were followed for 4 months with electrocardiographic assessment of the QTc interval and piperaquine drugs levels at 0, 4, 24 and 28 hours post dosing. The study, with a planned enrollment of 231 volunteers was suspended after only 6 weeks (69 volunteers enrolled) when 4 volunteers met a pre-specified cardiac safety endpoint with >500 ms QTcF prolongation. Effect peaked at approximately 4 hours after piperaquine dosing, and lasted 4-8 hours. Mean QTcF prolongation of 50ms over placebo was seen at Tmax on day 2. The study was halted after review by an independent data safety monitoring board. Given the utility of DHA-piperaquine as one of the few remaining effective antimalarials in Cambodia, until the clinical significance of the findings can be more thoroughly evaluated, compressed 2 day treatment courses are best avoided. Repolarization risk using conventional 3 day doses of DHA-piperaquine can be mitigated by fasting for 3 hours before/after administration. Avoiding repeated dosing, unintentional overdose, and coadministration of other QT prolonging medications is also recommended. Other potential safety interventions including EKG and electrolyte monitoring are rarely available in settings where malaria is endemic.

807

EXPLORING PHARMACOKINETICALLY GUIDED POTENTIAL ANTIMALARIAL COMBINATION THERAPY

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Lumefantrine (LUME) in combination with artemether is the current first-line therapy for treatment of uncomplicated *falciparum* malaria. All the currently recommended firstline antimalarial combinations include an artemesinin derivative as one of the therapeutic agent. The commercial

availability of artemisinins is scarce due to their natural biodistribution as well as tedious and costly extraction procedure. Also, resistance has been observed for even artemisinins now. There are few cases reported for development of resistance to present combinations. Clinical treatment failure has even been reported for the firstline combination artemether-lumefantrine. 99-411 and 97-78 are short acting candidate antimalarials presently under clinical trials in India. Thus, we assessed the *in vivo* pharmacokinetic compatibility of trioxane candidate antimalarial, 99-411 and 97-78 with longer half-life partner drug, LUME and explored their potential to be developed as an effective antimalarial combination treatment. The examination of 99-411 and 97-78 with LUME as futuristic antimalarial combination indicates that LUME co-administration significantly enhanced the exposure of 99-411, which may be beneficial for the therapeutic efficacy. However, LUME exposure reduced significantly when co-administered with 97-78 which was not significantly affected. LUME did increase the AUC, C_{max}, Tmax of co-administered 99-411, however, the plasma half-life was found to decrease. On exploring the mechanisms that may be responsible in this additive interaction, the intestinal permeability studies show that LUME significantly increased the effective permeability of 99-411 by almost 2 fold while no such effect of 99-411 on LUME was seen. The pharmacokinetic evaluation of the two combinations suggests that LUME with 99-411 seems to have therapeutic merit and it may be useful in delaying the development of resistance against both the drugs which is the major reason of antimalarial therapy failure world over.

808

THE P-TYPE CATION-TRANSPORTER ATPASE, PFATP4, IS A MULTIDRUG RESISTANCE ASSOCIATED GENE IN *PLASMODIUM FALCIPARUM*

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Efforts to control malaria are continuously challenged by the emergence of drug resistance. New drugs will thus be needed for treatment and control efforts aimed at eliminating this disease that causes 700,000 unnecessary deaths annually. Aminopyrazoles are a unique class of antimalarial compounds with potent activity against *Plasmodium falciparum* blood stage parasites which were identified in a cellular antiparasitic screen and whose mechanism of action is unknown. To investigate their mechanism of action we pressured parasites until they acquired low-level resistance to a representative member of the aminopyrazole series, GNF358, creating three independent resistant lines. The IC50 value of each clone was 3-10 fold greater than that of the parental Dd2 clone (148 nM). Whole-genome sequencing of the resistant lines showed that each had acquired independent mutations in a P-type cation-transporter ATPase, PfATP4 (PF3D7_1211900), a gene implicated in resistance to another, structurally unrelated, class of antimalarials, the spiroindolones which are currently in Phase II clinical trials. Not unexpectedly, GNF358-resistant lines also exhibited selective cross-resistance to the spiroindolones with IC50 values shifted more than 5-fold greater than that observed for the drug-sensitive parental line. Like the spiroindolones, we show that GNF358 inhibits gametocyte transmission as well as disrupts sodium homeostasis in the parasites. Additionally, transgenic parasite lines harboring directed mutations in PfATP4 also show cross-resistance with GNF358. Our data show PfATP4 plays an important role in cellular processes, can be inhibited by two distinct antimalarial pharmacophores and supports the recent observations that PfATP4 is a critical drug target.

809

SAFETY AND EFFICACY OF REPEATED ADMINISTRATION OF PYRONARIDINE-ARTESUNATE OR DIHYDROARTEMISININ-PIPERAQUINE VS ARTESUNATE-AMODIAQUINE OR ARTEMETHER-LUMEFANTHRINE OVER A TWO-YEAR PERIOD IN CHILDREN AND ADULT PATIENTS WITH ACUTE UNCOMPLICATED *PLASMODIUM S.P.* MALARIA AT NIANGOLOKO SITE IN BURKINA FASO

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ACTs have been recommended as first line treatment for uncomplicated malaria by WHO. However, the safety of repeated administration of the same drug to an individual is not documented.The present study aims to assess the safety and effica.cy of repeated administration of pyronaridine-artesunate (PA) or dihydroartemisinin-piperaquine (DHA-PQ) vs artesunate-amodiaquine (ASAQ) or artemether-lumefanthrine (AL) over a period of 2 year. The primary endpoints are to assess the non-inferiority of PA or DHA-PQP vs ASAQ or AL in term of the incidence rate of uncomplicated malaria in children and adults treated with repeated ACT therapy over a 2 year observation period and to assess the non-inferiority of PA or DHA-PQP vs ASAQ or AL in term of PCR corrected and uncorrected ACPR at D28 and D42 (WHO definitions 2009).We are carrying out a comparative, randomized, open label longitudinal clinical trial involving children and adults with uncomplicated *Plasmodium sp.* malaria. In the current study, at the enrolment, eligible participants are randomly assigned to one of the treatment arm to receive 3-day course of either DHA-PQ or ASAQ. They are then followed up for 42 days. Standard assessments for antimalarial efficacy and safety trials are conducted. Hematology, biochemistry, PK and 12 Lead ECG are done at various predefined timepoints. During their subsequent episodes, participants receive the same drug and will go through the same trial procedures as for the initial episode. The partial results from the first year of the follow up with DHA and ASAQ arms showed that 30 of 85 patients in the ASAQ arm compared to 20 of 85 in the DHA arm experienced 2 malaria episodes. The Adequate clinical and parasitological response (ACPR) by day 28 was 91.55 % in ASAQ arm compared to 100% in DHA-PQ arm. The total treatment failures with ASAQ by day 28 (not PCR corrected) were 8.45 % whilst failures in DHA-PQ arm were 0%. The 42 cure rates (not adjusted by PCR) were 69.88 % and 96.35 % in ASAQ and DHA-PQ arms respectively. Our preliminary results confirmed the two drugs are safe and indicated that in high transmission region the antimalarial efficacy of DHA-PQ was superior to that ASAQ. The study is ongoing to complete the entire sample size and follow the patients over a period of two years.

810

INTRANASAL ADMINISTRATION OF ARTESUNATE AND ERYTHROPOIETIN TO TREAT CEREBRAL MALARIA

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Despite treatment with anti-malarial drugs, patients die from cerebral malaria and too many are discharged with neurological deficits. In the long term, 1/4 of survivors have neurological and cognitive impairments years after exposure. Currently, treatment of malaria is based mainly on drugs such as artesunate. Erythropoietin antagonizes both the production and the effects of pro-inflammatory cytokines and limits perfusion-reperfusion injury in a variety of tissues. Systemic administration of recombinant human EPO (rhEPO) is protective in animal models for stroke, spinal cord injury, myocardial infarction, and ischemia-reperfusion among many others. Human trials with rhEPO have demonstrated tissue protective activities in stroke, multiple sclerosis, multi-system trauma requiring intensive care support similar to those observed in the preclinical models. We first reported the protective effect of rhEPO in murine cerebral malaria.

We also demonstrated that high doses Erythropoietin were safe for children suffering severe cerebral malaria. Use as adjunctive treatment associated with anti-malarial drug, Epo could help to de-crease the early mortality rate of severe malaria. Experimental malaria was induced in mice infected with *Plasmodium berghei* ANKA. Animal experiments were conducted in agreement with ethical and general rules for animal protection. Drugs were used at serial dilutions and administered either I.P. (control groups), using in-tranasal spray device, or dropwise after anesthesia. Mice were monitored for signs of CM, including body weight, hematocrit, ataxia, paralysis, deviation of the head, convulsions, and coma. Parasitemia were determined using Giemsa-stained thin blood films. Intranasal administration of artesunate was highly efficient to decrease blood *parasitemia* in *P. berghei* infected mice. The cure rate using 40mg/kg b.w; was 100% for two successive experiments (60 mice). Same efficacy was obtained when the artesunate dose was deacreased. This is the first evidence that artesunate and erythropoietin treatment could be administered via intranasal route.

811

THE MECHANISMS OF ACTION OF TWO NEW BENZOXABOROLES AGAINST *PLASMODIUM FALCIPARUM*

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There is an urgent need for new antimalarials, preferably with novel mechanisms of action. We have identified several boron-containing compounds with excellent *in vitro* potency and *in vivo* efficacy (AN3661: IC_{50} 37 nM against W2-strain *Plasmodium falciparum*, ED_{90} 0.3 mg/kg against murine *P. berghei*; AN6426: IC_{50} 196 nM, ED_{90} 7.44 mg/kg). AN3661, AN6426, and related compounds were tested for stage-specificity by incubation with test compounds for 8 h intervals across the parasite erythrocytic life cycle. They were also tested for inhibition of protein synthesis by comparing the incorporation of 14[C]Leu by parasites treated with test compounds or controls and for inhibition of plasmodial leucyl tRNA synthetase (LeuRS), an antimicrobial target of other benzoxaboroles, by monitoring 14[C]Leu incorporation in cytoplasmic extracts including exogenous tRNA. The trophozoite stage was most sensitive to AN3661 and AN6426. Dose-dependent inhibition of both protein synthesis and LeuRS activity was observed for AN6426, but not AN3661 or the control artemisinin, supporting different mechanisms for the different benzoxaboroles. To gain further insight into mechanisms of action we selected for *P. falciparum* with decreased sensitivity to AN3661 or AN6426 by culturing W2-strain parasites in step-wise increasing concentrations of the compounds and Dd2-strain parasites in one high concentration of AN3661. Cross-resistance was not seen between parasites selected with AN3661 and those selected with AN6426. Whole genome sequencing of multiple clones selected for resistance to AN3661 revealed several SNPs in a gene that codes for homologs of mammalian cleavage and polyadenylation specificity factor (CPSF; PF14_0364). Sequencing of parasites selected for resistance to AN6426 showed several SNPs in the editing domain of the predicted cytoplasmic LeuRS gene (PFF1095w). In summary, we offer strong evidence for unique antimalarial mechanisms of action for two benzoxaboroles, identifying two novel antimalarial drug targets. Further investigation of these novel benzoxaborole mechanisms is underway.

813

EFFICACY, SAFETY AND POPULATION-PHARMACOKINETICS OF THE ARTESUNATE-MEFLOQUINE (ASMQ) FIXED DOSE COMBINATION VERSUS ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN AFRICAN CHILDREN

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WHO guidelines recommend fixed-dose combinations of artemisin-based combination therapies (ACTs) to treat uncomplicated *Plasmodium falciparum* malaria. The use of the combination of AS with MQ (non-fixed and fixed) is well documented in Asia (high efficacy, good safety). In Africa, however, there is limited experience with the combination and no data regarding MQ pharmacokinetics in children treated with the fixed dose combination (FDC). Our objective was to evaluate the efficacy, safety and population-pharmacokinetics of fixed dose ASMQ in children. A randomized clinical study was conducted in children under 5 years of age in Kenya, Tanzania and Burkina Faso. It consists of a 3-day treatment period and 60-day follow-up. Patients received either ASMQ FDC or Artemether-Lumefantrine. The randomized clinical study is still ongoing. MQ data were obtained in 50 Kenyan patients and analysed using population pharmacokinetics (NONMEM®). Blood samples were collected before dosing at day (D) 0 and at 5 other time points on D0, D2, D3, D7 and 1 sample at randomly selected days during the follow-up visits (days 28 35 42, 49, 56 or 63). A two-compartment model best described MQ pharmacokinetics. The estimates and the variability (CV%) of the pharmacokinetic parameters were a systemic clearance of 0.20 L/h (40%), volumes of distribution of the central and peripheral compartment of 43.5 L (51%) and 24.2 L, an inter-compartmental clearance of 0.12 L/h, and an absorption rate constant of 0.17 h-1 (97%). No available covariates could explain the large inter-patient variability observed in the pharmacokinetic parameters, except for age on Ka. Average absorption time was 6.3 h (range: 1.4-27.4 h) and mean terminal half-life 14.1 days (9.3-46.5 days). MQ pharmacokinetics present large inter-patient variability in children treated with fixed dose regimen. Clearance and volume of distribution of MQ in children is lower than in adult patients, but the terminal elimination half-life and mean absorption time are of similar magnitude.

814

RELATIVE BIOAVAILABILITY OF CO-ADMINISTERED FORMULATIONS OF AZITHROMYCIN (AZ) MICROSPHERE AND CHLOROQUINE (CQ) TEST FORMULATION COMPARED WITH CO-ADMINISTERED IMMEDIATE RELEASE INDIVIDUAL AZ AND CQ TABLETS IN HEALTHY ADULT SUBJECTS

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The combination of AZ and CQ has shown synergistic activity against *Plasmodium falciparum in vivo* and *in vitro*. The better gastrointestinal (GI) tolerability of azithromycin (AZ) extended-release (ER) microsphere formulation (Zmax®) relative to AZ immediate-release (IR) formulation allows administration of a higher single dose of AZ with acceptable GI tolerability. CQ phosphate was formulated as an exploratory microsphere formulation to mask the bitter taste of CQ that could be co-administered with AZ ER for malaria treatment or prophylaxis. This Phase 1, open-label, single-dose, parallel-group study evaluated the relative bioavailability of co-administered AZ ER (2 g) and experimental microsphere formulation of CQ (620 mg base; Test Formulation) compared with co-administered IR individual commercial tablets of AZ (2 g) and CQ (600 mg CQ base; Reference Formulation) in 40 healthy adults. Subjects were confined to the Clinical Research Unit for 2 days with additional clinic visits on Days

3-5 following enrollment. Blood samples were drawn at pre-specified times up to 96 hours post dosing and analyzed to determine AZ and CQ concentrations. Noncompartmental analysis of concentration-time data was used to calculate pharmacokinetic parameters of maximal observed drug concentration (Cmax), time to Cmax (Tmax), and area under the drug concentration-time curve from time zero to the time of the last quantifiable concentration (AUClast). ANOVA was used to compare natural log-transformed AUClast and Cmax with group (main effect) and log weight (covariate). Safety evaluations included monitoring of adverse events (AE), clinical laboratory tests, and vital signs. All enrolled subjects completed the study. The relative bioavailability AUClast values (90% CI) of serum AZ and plasma CQ (Test Formulation) were 73.79% (63.99%-85.08%) and 105.47% (93.39%-119.11%), respectively, compared to the Reference Formulation. The AE profile and GI tolerability were better for the Test formulation compared to the Reference Formulation. No safety issues were identified in either treatment.

815

TEACHING AN OLD DRUG NEW TRICKS: NOVEL AMODIAQUINE ANALOGUES WITH POTENT ANTIPLASMODIAL ACTIVITY AND A POTENTIALLY IMPROVED SAFETY PROFILE

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The devastating morbidity and high mortality associated with malaria infection, coupled with the development of drug resistance to new treatment regimens almost as soon as they are introduced, underline the imperative for sustained research into new antimalarial agents. It is important that these new agents are not only efficacious but also safe. Drug discovery is a costly and time consuming process. One of the approaches adopted to circumvent or mitigate against this bottleneck is the use of existing drugs as lead compounds upon which suitable chemical modifications can be carried out. The 4-aminoquinoline antimalarial amodiaquine exhibits excellent activity against both sensitive and resistant *Plasmodium falciparum* strains. Its use is, however, restricted due to severe hepatotoxicity and agranulocytosis. This toxicity is attributed to metabolic activation to the quinone imine and aldehyde metabolites, respectively. These reactive metabolites bind to and disrupt the function of cellular macromolecules. We hypothesized that masking the functionality responsible for the formation of these reactive metabolites in the form of a benzoheterocyclic system would block bioactivation while retaining potent antiplasmodial activity against both drug sensitive and resistant strains of *P. falciparum*. As a proof of concept, novel benzoheterocyclic analogues were synthesized and subjected to glutathione trapping studies using electrochemical oxidation online with electrospray ionization mass spectroscopy (EC-ESI/MS) to give an indication of the potential for reactive metabolite formation. The results confirmed that the potential to form the quinone imine was eliminated but potent antiplasmodial activity was retained. In conclusion, these results demonstrate that benzoheterocyclic analogues based on amodiaquine are promising leads in the discovery of novel safer aminoquinoline antimalarials.

816

TRANSMISSION BLOCKING PROPERTIES OF ANTIMALARIALS

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Malaria transmission is critically dependent on sexually differentiated gametocytes that develop in the bloodstream of the human host and are infectious to the mosquito vector. These gametocytes are not sensitive to the vast majority of marketed antimalarials and patients treated for clinical malaria may still carry gametocytes and transmit the disease. Successful elimination of malaria depends on the generation of new intervention tools that target the transmission stages of the parasite. We have evaluated the transmission-blocking potential of components of Artimisinin based Combination Therapy and of a number of candidate drugs from the Medicines for Malaria Venture R&D portfolio. To this end, we tested compound effects against *Plasmodium falciparum* gametocytes in a newly developed pLDH assay that monitors gametocyte metabolic activity. In addition, we developed an assay to quantify gametogenesis capacity and have modified the protocol for a Standard Membrane Feeding Assay (SMFA) in order to establish precise dose response measurements on sporogonic development in the mosquito. Our assays revealed distinct mechanisms of action for different compounds. Artemisinins, and in particular the active metabolite dihydroartemisinin, show activity against gametocytes, which results in a partial block in gamete formation and a full block in oocyst development. Quantitative measurements show that DHA is 30-fold more potent against asexual parasite stages than against oocyst formation in the mosquito. Other compounds, such as pyronaridine, do not affect gametocyte pLDH activity but block oocyst development, suggesting they act directly against the sporogonic stages in the mosquito midgut. Lastly, we tested a number of compounds from the MMV drug discovery portfolio. The majority of compounds in early clinical development exhibit multi-stage activity and block transmission. Our data indicate differential effects on oocyst intensity and oocyst prevalence (number of infected mosquitoes), which depend on the parasite exposure and specific mechanism of action. Our studies identified a small number of compounds for which the efficacy is relatively independent of the parasite exposure.

817

LEAD OPTIMIZATION OF BROAD-SPECTRUM ANTIMALARIAL ACRIDONES

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We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced *Plasmodium* infection in addition to efficacy against blood stage parasites. An aggressive optimization process not only expanded our chemical library but more importantly produced a new lead candidate with significantly improved efficacy, both *in vitro* and *in vivo*. The new lead candidate also exhibits potent activity against atovaquone-resistant parasites. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

818

PHASE 2 RANDOMIZED PROOF OF CONCEPT STUDY EFFICACY TRIAL COMPARING AN INVESTIGATIONAL AMINOQUINOLINE ANTIMALARIAL (AQ-13) TO COARTEM IN MALIAN MALES WITH UNCOMPLICATED MALARIA

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Although artemisinin-combination therapies (ACTs) are the recommended first-line treatment for uncomplicated *Plamodium falciparum* malaria, there

is increasing concern about artemisinin resistance because of prolonged parasite clearance times in Southeast Asia. For this reason, it would be extremely valuable to have alternatives to ACTs that were effective against chloroquine(CQ)-resistant *P. falciparum*, economical and could be given orally. Our previous studies have shown that aminoquinolines (AQs) with modified side chains such as AQ-13 are active against CQ- and multi-resistant *P. falciparum in vitro* and in a squirrel monkey model of human infection with CQ-resistant *P. falciparum*, are as safe as CQ in healthy human subjects and have similar pharmacokinetics. This study randomizes adult Malian males (≥18 years of age) with uncomplicated *P. falciparum* malaria to receive 1,750 mg of AQ-13 orally over 3 days or the currently recommended dose of Coartem (480 mg artemether + 2,880 mg lumefantrine) orally over 3 days. The major endpoint is treatment failure (failure to reduce asexual *parasitemia* to <25% of baseline by day 3, clear all asexual parasites by day 7 or recurrent infection with the same parasite genotype between days 8 and 42). Secondary endpoints include clinically apparent adverse events, parasite and fever clearance times, time to recurrent infection, pharmacokinetic parameters for AQ-13 and prolongation of the QT (QTc) interval. Tertiary endpoints include the pharmacokinetics of AQ-13 metabolites and the occurrence of pruritus in persons receiving AQ-13. Based on a start date in June, we expect that the initial 66 subjects will have been enrolled by October or earlier and that the initial results should be available for presentation in November.

819

DEVELOPMENT OF A HUMANIZED PSEUDO-LIVER NOD/SCID MOUSE MODEL OF DRUG METABOLISM

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Humanized mouse models are a powerful tool for direct investigation of preclinical drugs and human disease. We are currently developing a humanized SCID mouse model to assess the hemolytic potential of candidate antimalarial drugs in the context of glucose-6-phosphate dehydrogenase deficiency ($G6PD^-$), the most common human enzymopathy which results in hemolytic anemia in patients treated with drugs such as primaquine (PQ). We previously reported on the development and validation of a human (hu)RBC-SCID mouse model in which NOD/SCID mice are given daily transfusions of human red blood cells from $G6PD^-$ donors. Our efforts aim to test 8-aminoquinoline antimalarial drug candidates derived from the parent drug PQ for hemolytic toxicity. A major limitation of humanized mice is that they retain physiological characteristics of an animal system that are not identical to a human system such as cytochrome p450 (CYP)-dependent drug metabolism. It is therefore difficult to predict pathways of CYP-dependent metabolism based on our huRBC-SCID model because the variation in functionality of CYP family members between mice and humans is incompletely understood. The metabolic species responsible for toxicity in the 8AQ class and their associated pathways are also not fully understood. Ideally, humanized livers could be combined with the huRBC-SCID model to assess the role of human metabolic activity in the background of hemolytic toxicity induced by antimalarials. Since technical challenges limit this approach, we propose establishing s.c. tumors in NOD/SCID mice arising from the HepG2 human hepatoma cell line as a viable alternative. HepG2 cells retain some characteristics of a normal human liver and can be transfected with human CYP to examine the role of an individual CYP in PQ metabolism. To determine relative HepG2 CYP expression both *in vitro* and *in vivo* compared to normal liver, RT-qPCR was performed to analyze expression of CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 relative to β-actin in HepG2 cells, HepG2 tumors established in NOD/SCID mice and normal human liver. Results show that CYP expression is reduced in both HepG2 cells *in vitro* and *in vivo* compared to normal liver, demonstrating that this cell line may be a suitable vehicle for establishing a pseudo-liver in our $G6PD^-$ model. Ongoing studies are aimed at expressing CYP2D6 and CYP3A4 in pseudo-livers to evaluate their role in PQ metabolism.

820

SONTOCHIN AS A GUIDE FOR DEVELOPMENT OF CHLOROQUINE REPLACEMENT DRUGS

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Sontochin was the original chloroquine replacement drug, arising from research by Hans Andersag two years after chloroquine ("Resochin") had been abandoned due to the mistaken perception that it was too toxic for human use. We found that sontochin, i.e., 3-methyl-chloroquine, retains significant activity against chloroquine resistant strains of *Plasmodium falciparum in vitro*. As a result we began to explore sontochin analogs, "pharmachins", with alkyl or aryl substituents at the 3-position of the 4-aminoquinoline core. Modified with an aryl substituent in the 3-position of the 7-chloroquinoline ring, PH-203 exhibits low nanomolar IC_{50} values against drug sensitive and multidrug resistant strains and *in vivo* efficacy against patent infections of *P. yoelii* in mice that is superior to chloroquine. With sontochin and PH-203 as structural leads for optimization we have continued to elaborate a library of pharmachins varying the aryl substituent at the 3-position while optimizing the scaffold for *in vitro* activity and *in vivo* efficacy against malaria in a murine model of the disease. As part of the test cascade we characterized selected molecules for pharmacokinetics and for off-target effects including cytotoxicity and blockade of the human Herg channel. We have also profiled our frontrunners against a broad range of targets including human receptors, ion channels, transporters, enzymes and second messengers to guide the down-selection process. Our results show that novel 3-position aryl pharmachins represent potential chloroquine replacement drugs for treating malaria in humans.

821

OPTIMIZATION OF A LIGASE DETECTION REACTION-FLUORESCENT MICROSPHERE ASSAY FOR THE CHARACTERIZATION OF RESISTANCE-MEDIATING POLYMORPHISMS IN AFRICAN SAMPLES OF *PLASMODIUM FALCIPARUM*

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Genetic polymorphisms in the malaria parasite *Plasmodium falciparum* mediate alterations in sensitivity to important antimalarial drugs. Surveillance for these polymorphisms is helpful in assessing the prevalence of drug resistance and designing strategies for malaria control. Multiple methods are available for the assessment of *P. falciparum* genetic polymorphisms, but these suffer from low throughput, technical limitations, and high cost. We have optimized and tested a multiplex ligase detection reaction-fluorescent microsphere (LDR-FM) assay for the identification of important *P. falciparum* genetic polymorphisms. For 83 clinical samples from Kampala, Uganda, a region where transmission intensity and infection complexity are both high, DNA was extracted from dried blood spots, genes of interest were amplified by PCR, amplicons were subjected to multiplex ligase detection reactions to add bead-specific oligonucleotides and biotin, fragments were hybridized to magnetic beads, and polymorphism prevalences were assessed fluorometrically in a multiplex format. A total of 19 alleles from the pfcrt (codons 72-76 CVMNK, CVIET, or SVMNT), pfmdr1 (N86Y, Y184F, S1034C, N1042D, D1246Y), pfmrp1 (I876V), pfdhfr (N51I, C59R, S108N or T, I164L) and pfdhps (S436A, A437G, K540E, A581G, A613S) genes were analyzed by LDR-FM and restriction fragment length polymorphism (RFLP) analysis.

Considering samples with results from both assays, concordance between the assays was good, with 78-100% of results identical at individual alleles, most non-concordant results differing only between a mixed and pure genotype call, and full disagreement at individual alleles in only 0-3% of results. We estimate the LDR-FM assay to offer much higher throughput and lower cost compared to RFLP analysis. Our results suggest that the LDR-FM system offers an accurate high throughput means of classifying genetic polymorphisms in field samples of *P. falciparum*.

822

SAMPLING DESIGN FOR THE PARASITE CLEARANCE ESTIMATOR: TWO COMPLEMENTARY METHODOLOGICAL APPROACHES

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The key pharmacodynamic measure of the artemisinin derivatives is the rate of parasite clearance in the days following treatment. Frequent assessments of the parasite density are needed to define this rate. The aim of this work was to apply two different methodologies to derive sampling schedules from which parasite clearance could be defined using the WWARN Parasite Clearance Estimator (PCE). The first approach used robust T-optimal design methodology to allow for discrimination across models that best describe an individual patient's parasite-time profile. The design was based on the constraint of no more than six samples per patient within 48 hours of initial treatment. The design was evaluated with a simulation-estimation procedure. The second approach selected 2744 real parasite-time patient profiles with 6 hourly counts in the first 48 hours. Bootstrapping was used to estimate the sampling distribution of half-lives (HL) from sampling schedules that excluded data from at least one of the 6-hourly sampling time-points (including the scheme 0, 6, 12, 24, then every 12 hours (A1)). A simulation study was performed to investigate 16 schemes, including scheme A1 and the T-optimal scheme. The T-optimal sampling times (sampling windows) were: 0 (0 to 2), 5.8 (4.0 to 6.0), 9.9 (8.0 to 12.0), 24.8 (24.0 to 24.9), 36.3 (34.0 to 38.0) and 48 (45.0, 48.0) hours post initial treatment. The simulation-estimation procedure showed that the design supported selection of the appropriate model for parasite clearance rate constant estimation. For the data-based approach, scheme A1 consistently performed the best, for a range of HLs. Of the reduced schemes, one with measurements at times[window] of 0[0-2], 6[4-8], 12[10-14], 24[22-26], 36[34-36], 48[46-50], or 6, 7, 24, 25, then every 24 hours (+ the subsequent hour) is recommended. Stopping sampling at 48 hours had little effect on the estimation of HL. The two methodologies produced consistent findings. The derived designs require further validation, but should be considered for future studies that intend to use the PCE.

823

IMPACT OF ARTEMISININ BASED COMBINATION THERAPY (ACTS) REPEATED TREATMENT ON THE PREVALENCE OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MOLECULAR MARKERS, *PFCRT* AND *PFMDR1*

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Based Combination Therapy (ACTs) are currently used as the malaria first-line treatment in most endemic countries. The aim of this study was to assess the impact of repeated treatment with AS + AQ and AR-L on *Pfcrt* and *Pfmdr1*, in a 3 years randomized clinical trial in Bougoula (Mali). We use WHO 28-day standard in-vivo protocol. Overal 521 blood spoted filter papers were analyzed; mutations frequencies on *Pfcrt* and *Pfmdr1* genes were compared before and after intervention. In the AS + AQ arm we observed a base line frequency of 41.6% against 77.1% for *Pfmdr1-86Y* during the first episode and > 93% in the second, third and fourth episodes of malaria. For the *Pfcrt76T gene* we observe a baseline frequency of 58.9% against 88% during the first episodes and > 93% in the next episodes. For the AR-L arm's, we obtained a baseline frequency of 41.6% against 6.2%, 18.2%, 7.1% and 0% on *Pfmdr186Y gene* for episodes 1, 2, 3 and 5 respectively. Concerning *Pfcrt76T* gene the base line frequency was 58.9% against 59.1%, 75% and 88.8% for episodes 1, 2 and 3 respectively. This study demonstrate that there is a significant increase in *Pfmdr-86Y*, and *Pfcrt-76T* mutants after treatment with AS + AQ and a significant decrease of *Pfmdr1* mutations after treatment with AR-L. Despite the presence of artemisinin,the CTAs select the molecular markers of resistance to the partner molecule.

824

POOLED DEEP SEQUENCING OF *PLASMODIUM FALCIPARUM PARASITEMIAS*: AN EFFICIENT AND SCALABLE TOOL TO QUANTIFY PREVAILING MALARIA DRUG-RESISTANCE GENOTYPES

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Molecular surveillance for drug-resistant malaria parasites requires efficient, timely, and scalable methods in order to provide actionable data. Genotyping parasite populations using second-generation sequencing may provide these data efficiently. We designed and validated a protocol to quantify the frequencies of mutant alleles associated with sulfadoxine-pyrmethamine resistance in *Plasmodium falciparum* genes dhfr and dhps in mixed *parasitemia*s using 454 sequencing. We applied this new protocol to field isolates collected from a cohort of 50 Tanzanian children with uncomplicated *falciparum* malaria, and compared it on accuracy and cost to standard genotyping methods that employ Sanger sequencing with or without statistical inference of allele frequencies. In validation experiments with a mixture of parasite strains 3d7 and V1/S, the 454 sequencing protocol accurately quantified dhfr and dhps allele frequencies. Using Sanger sequencing with statistical inference, the frequencies of mutant alleles in dhfr were 78.9% (dhfr51I), 80.5% (dhfr59R), 86.2% (dhfr108N), and 0 (dhfr164L); mutation frequencies in dhps were 58.5% (dhps437G), 51.1% (dhps540E), and 1.1% (dhps581G). 454 sequencing of pooled gDNA generated 91,157 and 92,638 reads of dhfr and dhps, respectively, which estimated mutant allele frequencies of 70.9% (dhfr51I),

84.6% (dhfr59R), 90.2% (dhfr108N), 0 (dhfr164L), 55.1% (dhps437G), 57.9% (dhps540E), and 6.2% (dhps581G); these estimates were highly correlated (>98%) with frequencies estimated by traditional methods. 454 sequencing obviated most molecular steps in traditional sequencing methods, and because of this would be cost-saving to generate allele frequencies for parasite population sizes larger than 50. This genotyping method based upon second-generation sequencing can efficiently and reproducibly estimate parasite allele frequencies within populations of *P. falciparum*. This method would be rapid and cost-effective for molecular epidemiologic studies of parasite genotypes associated with transmission, vaccine efficacy, and drug resistance.

825

GENOMIC EPIDEMIOLOGY OF ARTEMISININ RESISTANCE ON THE CHINA-MYANMAR BORDER

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Emerging artemisinin resistance in Southeast Asia poses a significant risk to malaria control and eradication goals, including China's plan to eliminate malaria within its borders by 2020. Yunnan, China's only province with endemic *Plasmodium falciparum* malaria transmission, borders Myanmar, Vietnam and Laos, and is the key focus of the national malaria elimination program. Parasites from this region have shown decreased *in vitro* susceptibility to artemisinin and delayed parasite clearance after artemisinin treatment. Understanding the genetic basis of artemisinin resistance and identifying specific genetic *loci* associated with this phenotype is crucial for effective surveillance and containment of resistance. Parasites collected from 200 clinical trial participants from three field sites near the Myanmar border were genotyped using a *P. falciparum*-specific single nucleotide polymorphism (SNP) microarray. SNP profiles were examined for signatures of recent positive selection and prevalence of validated and candidate molecular markers of drug resistance. Population structure in each parasite population was evaluated by Principal Component Analysis and the program Structure, which uses a Bayesian framework for estimating sub-structure. Results of these genomic analyses will be discussed in the context of recent genome-wide association studies of genetic *loci* associated with artemisinin resistance. This study verifies the utility of DNA microarrays for large-scale parasite molecular epidemiology and will aid in the validation of candidate artemisinin resistance markers.

826

MONITORING THE EFFICACY AND SAFETY OF THREE ARTEMISININ COMBINATIONS THERAPIES (ACT) IN SENEGAL: RESULTS FROM TWO YEARS SURVEILLANCE

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Malaria is a major public health problem in developing countries. The malaria control strategy includes the case management of clinical case. The obstacle of the case management of clinical malaria is the emergence and the spread of malaria drugs resistance. To cope with this situation WHO recommended the artemisinin combination therapies (ACT). After the scaling up of ACT in Senegal and the and the recent appearance of a decreased susceptibility of Pf to artemisinin based combination therapy in Asia, it becomes necessary to monitor the use of ACT in Africa west. The study was carried out in the transmission period of 2011 and 2012 in two health districts in Senegal. Study end points included (i) PCR corrected adequate clinical and parasitological response (ACPR) at day 28, (ii) ACPR at days 35 and 42, (iii) parasites and fever clearance time, (iv) incidence of adverse events and patients biological profile at day 7. The WHO 2003 protocol for antimalarial drug efficacy evaluation was used to assess each outcome. Three ACT were evaluated: dihydro-artemisinine-piperaquine DHAPQ (Duocotexcin*), artemether-lumefantrine AL (Coartem*) and artesunate-amodiaquine ASAQ. Overall, 534 patients were randomized to receive either DHAPQ (n=176), AL (n=178) and ASAQ (n=180). PCR corrected ACPR at day 28 was at 99.41% in ASAQ group while that was at 100% in DHAPQ and AL group (p=0.37). Therapeutic efficacy was at 100% in DHAPQ and AL group versus 99.37% in ASAQ group at day 35 (p=0.37). At day 42 ACPR at 100% was obtained in the DHAPQ and AL group versus 99.27% in ASAQ group (p=0.36).The three treatments were well tolerated with similar clinical and biological profile. In conclusion, Dihydro-Artémisinine-Pipéraquine (DHAPQ), Artemether-lumefantrine (AL) and Artesunate-Amodiaquine (ASAQ) are effectious and well tolerated for malaria treatment in Senegal mainly in West Africa in spite of the recent appearance of a decreased susceptibility of Pf to artemisinin based combination therapy in Asia.

827

EFFICACY OF THREE REGIMENS FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CAMBODIA

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Resistance to anti-malarial drugs, including artemisinin combination therapies is a growing problem. We assessed the efficacy of three therapies for uncomplicated *Plasmodium falciparum* malaria in Trapeang Prasat, Oddarmeanchey Province, Cambodia bordering Thailand. Sixty-three subjects with uncomplicated *P. falciparum* malaria received directly observed therapy with 12 mg/kg artesunate and 25 mg/kg mefloquine (A/M, over three days), up to a maximum dose of 600 mg artesunate/1250 mg mefloquine; 77 subjects received dihydroartemisinin-piperaquine (DP); 71 subjects received Malarone. Subjects were followed for 42 days or until recurrent *parasitemia* was observed. We used PCR genotyping of msp1, msp2, and glurp to distinguish treatment failure from new infections andtreatment failure rates at days 28 and 42 were analyzed with both per protocol and Kaplan-Meier. Real Time PCR was used to measure the copy number of the pfmdr gene and standard 48 hour isotopic hypoxanthine incorporation assays was used to measure IC_{50} for anti-malarial drugs. Fifty-two%, 62.3%, and 62.3% infected subjects were still parasitemic on day 3 respectively. The crude treatment failure rates at 28 days were 8%, 1.3%, and 4.3% respectively and at 42 days were 19%, 3%, and 6% respectively. Treatment failure was associated with increased pfmdr1 copy number for A/M and DP groups only; no difference noted for the malarone group. One subject in the A/M arm acquired new *P. falciparum* infection; the rest of recurrences were thought to be recrudescence. All four recurrent cases in the Malarone arm were found to be of cytochrome b wild type. In conclusion, the results support other studies that artesunate-mefloquine combination therapy continues to worsen in Northern Cambodia, even in the "tier 2 region," outside of containment zone 1 where A/M resistance was first described. It is unclear whether the treatment failures are due solely to mefloquine resistance or to artesunate resistance as well.

828

SEQUENCE AND COPY NUMBER POLYMORPHISMS IN THE MULTIDRUG RESISTANCE TRANSPORTER 1 *(PVMDR1)* GENE OF *PLASMODIUM VIVAX* PARASITES FROM VANUATU AND THE SOLOMON ISLANDS

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In 2008, artemisinin-based combination therapy (ACT) was introduced as first-line treatment for confirmed cases of uncomplicated *Plasmodium falciparum* and *P. vivax* malaria in Vanuatu and the Solomon Islands, replacing chloroquine (CQ) and sulfadoxine-pyrimethamine (SP). The establishment of baseline drug resistance profiles allows for monitoring of the emergence of resistance to ACTs. Sequence polymorphisms in *pvmdr1* have been implicated in resistance to CQ and amodiaquine, while copy number polymorphisms in *pvmdr1* have been associated with resistance to mefloquine and lumefantrine. Using samples collected during therapeutic efficacy studies conducted in Epi Island, Vanuatu and Malaita Province, Solomon Islands, we investigated copy number and sequence polymorphisms in the *pvmdr1* gene of *P. vivax*. Results indicated that the majority of *P. vivax* parasites exhibited amino acid substitutions Y976**F** and F1076**L,** but retained only one copy of *pvmdr1*. This indicates *P. vivax* parasites in these islands are resistant to CQ and amodiaquine and a change of malaria treatment policy was timely.

829

ASSESSING THE COST-BENEFIT EFFECT OF A *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MUTATION ON PARASITE GROWTH *IN VITRO*

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Plasmodium falciparum mutations associated with antimalarial resistance may be beneficial for parasites under drug pressure, although they may also cause a fitness cost. We herein present an *in vitro* model showing how this combined effect on parasite growth varies with the drug concentration and suggest a calculated drug specific cost-benefit index, indicating the possible advantage for mutated parasites. We specifically studied *pfmdr1* D1246Y in relation to amodiaquine resistance. Susceptibilities to amodiaquine, desethylamodiaquine and chloroquine, as well as relative fitness were determined in two modified isogenic *P. falciparum* clones only differing in the *pfmdr1* 1246 position. Data were used to create a new comparative graph of the relative growth in relation to the drug concentration and to calculate the ratio between the benefit of resistance and the fitness cost. Results were related to an *in vivo* allele selection analysis after amodiaquine or artesunate-amodiaquine treatment. *Pfmdr1* 1246Y was associated with decreased susceptibility to amodiaquine and desethylamodiaquine, but at a growth fitness cost of 11%. Mutated parasites grew less in low drug concentrations due to a predominating fitness cost, but beyond a breakpoint concentration they grew more due to a predominating benefit of increased resistance. The cost-benefit indexes indicated that *pfmdr1* 1246Y was most advantageous for amodiaquine exposed parasites. In vivo a first drug selection of mutant parasites followed by a fitness selection of wild-type parasites supported the *in vitro* data. In conclusion, this cost-benefit model may predict the risk for selection of drug resistance mutations in different malaria transmission settings.

830

THE IMPACT OF MALARIA CONTROL INTERVENTIONS ON ANTIMALARIAL DRUG RESISTANCE: MEASURING THE ROLE OF CHANGES IN MULTIPLICITIES OF INFECTION

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To ensure effective malaria control strategies, it is important to investigate the impact of changing transmission on markers of antimalarial drug resistance, and thus genotypic multiplicity of infection. Genetic markers are often used to monitor antimalarial drug resistance, while multiclonal infections are largely ignored. However, interventions that reduce transmission intensity may also reduce the mean multiplicity of infection (MOI). The interpretation of changes in the prevalence of genetic markers in the face of changing MOIs is problematic, since prevalence is a function of the MOI. A Bayesian model, which uses a sampling algorithm, was developed to estimate genotype frequencies, which are comparable across different MOIs, and applied on a concrete study in an area with changing transmission: in 2003, an increase in prevalence of Pfdhfr wild type alleles after the introduction of insecticide treated nets (ITNs) in a village in Tanzania was reported, concurrent with a drop in the mean MOI. This paper addresses a major question: Did the decrease in the mean MOI alone result in changes in prevalence? Or did the differences reflect an increase in antimalarial susceptibility? Analyses based on statistically estimated genotype frequencies were compared with replicate analyses based on prevalence. Only small differences in the two approaches were detected, and they did not change the conclusion that the prevalence of susceptible alleles of Pfdhfr did increase after the introduction of ITNs. The authors' original proposal that 'lowering the level of transmission may be a method to indirectly increase sulfadoxine/pyrimethamine susceptibility by lowering drug use and, thus, drug pressure,' was substantiated, and the possibility that the increase in prevalence was a mere side effect of the drop in MOI ruled out.The new model has important implications for the surveillance of resistance in areas of decreasing malaria prevalence: by analysing frequency, estimates of resistance can be compared, and the confounding effect associated with changes in the MOI eliminated.

831

EFFICACY OF A THREE-DAY ARTESUNATE-MEFLOQUINE COMBINATION FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN MAE HONG SON, KANCHANABURI, UBONRATCHATHANI AND SURIN PROVINCES THAILAND IN 2011-2012

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Current first-line treatment for *Plasmpdium falciparum* in Thailand is a 3-day artesunate-mefloquine combination. Study conducted in In 2009, the efficacy of the artesunate-mefloquine combination declined to 87-92.7% in 4 sentinel sites. In 2010 the efficacy in 4 sentinel sites revealed

efficacy of the 3-day artesunate-mefloquine combination between 90.9-98.0%). In view of the threat from *falciparum* parasites developing resistance to ACTs recently, monitoring the efficacy of the first line treatment to generate valuable information for updating the national treatment policy is critical. This study was undertaken to assess the efficacy and safety of artesunate-mefloquine for the treatment of uncomplicated *P. falciparum* malaria in Mae Hong Son, Kanchanaburi, Ubonratchathani and Surin provinces in Thailand Antimalarial drug efficacy trials will be conducted in 4 sentinel sites in Thailand for uncomplicated *falciparum* infection. The participants will be febrile patients aged 6 months and above with confirmed uncomplicated *P. falciparum* infection. Falciparum malaria patients will be treated with artesunate (4 mg/kg over 3 days) co-administered with mefloquine (15 mg/kg on day 1 and 10 mg/kg on day 2). Clinical and parasitological parameters will be monitored over a 42-day follow-up period to evaluate drug efficacy for *falciparum* malaria. The study will be conducted from March to December, 2012. The results of this study will be used to assist the Ministry of Public Health of Thailand in assessing the current national treatment guidelines for uncomplicated *P. falciparum* malaria. Completed follow-up uncomplicated *P. falciparum* patients include in the per protocol analysis were 8, 43, 8, and 2 in Mae Hong Son, Kanchanaburi, Ubonrajchathani and Surin province respectively. According to the WHO criteria LCF (PCR corrected) that found in following province were 0, 8, 0, 0 respectively. The cure rate (ACPR) in the following province were 100%, 72.42%, 87.5% and 100% and day 3 positive were 0%, 55.81%, 12.5% and 0% respectively. In conclusion, the efficacy of artesunate-mefloquine has declined in Kanchanaburi province. Continuing monitoring drug efficacy in this area should proceed and changing of anti-malarial drug regimen are consider.

832

PERCEPTION OF MALARIA RISK IN A SETTING OF REDUCED MALARIA TRANSMISSION: A QUALITATIVE STUDY IN ZANZIBAR

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Malaria transmission has declined dramatically in Zanzibar in recent years. Continuing use of preventive measures such as long-lasting insecticidal-treated nets (LLINs), and use of malaria rapid diagnostic tests (RDTs) are essential to prevent malaria resurgence. This study employed qualitative methods to explore community perceptions of malaria risk and adherence to prevention measures in two districts in Zanzibar. Key informant interviews with 24 primary health care providers and 24 focus group discussions with local residents in Zanzibar districts Wete and Central were conducted during April and May 2012 focusing on perception of malaria risk, current preventive practices used, reasons for using preventive practices and effective strategies for malaria control. Health care providers and residents appear to be aware of the decreasing incidence of malaria. Both groups continue the use of malaria preventive practices in this low and seasonal transmission setting. The most important preventive measures identified were LLINs, indoor residual spraying (IRS), and education. Barriers to malaria prevention include: lack of staff at clinics, insufficient number of LLINs distributed, and inadequate malaria education. Reasons for continued use of preventive practices include: fear of malaria returning to high levels, presence of mosquitoes during rainy seasons, and concern about local cases from other villages or imported cases from mainland Tanzania. Mosques, clinics, schools and community meetings were listed as most important sources of education. However, residents express the desire for more education. Health care providers and residents generally reported consistent use of malaria preventive measures. However, maintaining and continuing to reduce malaria transmission will require ongoing education for both health care providers and residents to reinforce the importance of using preventive measures. Successful efforts to reduce malaria in Zanzibar will be jeopardized if residents believe that they are no longer at risk for malaria. In future studies, a year-round evaluation of the perception of malaria risk and use of preventive measures will inform the timing of education and prevention strategies for sustained malaria control.

833

FEBRILE ILLNESS MANAGEMENT IN CHILDREN UNDER FIVE YEARS OF AGE: A QUALITATIVE PILOT STUDY ON PRIMARY HEALTH CARE WORKERS PRACTICES IN ZANZIBAR

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In Zanzibar, malaria prevalence dropped substantially in the last decade and presently, most febrile patients seen in primary health care facilities (PHCF) test negative for malaria. The availability of RDTs allows rural health workers to reliably rule out malaria in fever patients. However, additional diagnostic tools to identify alternative fever causes are scarce, often leaving RDT negative patients without a clear diagnosis and management plan. This pilot study aimed to explore health workers' practices with febrile children and identify factors influencing their diagnostic and management decisions in non-malarial fever patients. Semi-structured key informant interviews were conducted with 12 health workers in six PHCFs in North A district, Zanzibar, April-June 2011. Interviews were coded using Atlas.ti to identify emerging themes that play a role in the diagnosis and management of febrile children. The following themes were identified: 1) Health workers use caregivers history of illness and RDT results for initial diagnostic and management decisions, but suggest caregivers need more education to prevent late presentation and poor health outcomes; 2) There is uncertainty regarding viral versus bacterial illness and health workers feel additional point-of-care diagnostic tests would help with differential diagnoses; 3) Stock-outs of medications and limited caregivers' resources are barriers to delivering good care; 4) Training, short courses and participation in research as well as; 5) Weather also influence the diagnostic decision-making. In conclusion, this pilot study found that health workers in Zanzibar use caregiver history of fever and results of malaria RDTs to guide management of febrile children. However, since most febrile children test negative for malaria, health workers believe additional training and point-of-care tests would improve their ability to diagnose and manage non-malarial fevers. Educating caregivers on signs and symptoms of febrile illness, as well as the introduction of additional tests to differentiate between viral and bacterial illness, would be important steps to get children to PHCFs earlier and decrease unnecessary antibiotic prescribing without compromising patient safety. More research is needed to expand our understanding of what would improve fever management in other resource-limited settings with decreasing malaria.

834

PLASMODIUM VIVAX HIGH-THROUGHPUT-LOOP MEDIATED ISOTHERMAL AMPLIFICATION (HTLAMP): IMPROVING THE DIAGNOSTIC TOOLKIT FOR MALARIA ELIMINATION

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Plasmodium vivax is the most geographically widespread of the *Plasmodium* species and poses formidable challenges to achieve elimination. Loop-mediated isothermal amplification (LAMP) holds potential as a sensitive molecular diagnostic technique for the identification of sub-patent malaria infection. However, its deployment is currently limited by the absence of a sufficiently sensitive species-specific assay for *P. vivax* infection, and by the absence of a platform suitable for high-throughput analysis in the field. Here we describe a new high-throughput, colourimetric, field applicable *P. vivax* LAMP(htLAMP) assay targeting the *P. vivax* mitochondrial COX1 gene. The assay was adapted to a 96-well plate colourimetric high-throughput format, and evaluated for sensitivity and specificity. The assay was performed in a 65^0 C waterbath with visually detectable colour-change results confirmed in an ELISA plate

reader. A *P. vivax* sample of known *parasitemia* (based on real-time PCR) was used to prepare a two-fold dilution series to establish the limit of detection of the assay. HtLAMP was then compared to nested PCR-based diagnosis on bloodspots extracted using saponin and chelex-based rapid DNA extraction protocols, to establish sensitivity and specificity. The limit of detection of the *P. vivax* htLAMP assay was 1.3 parasites/µL of packed red blood cells on DNA extracted from whole blood. The assay did not cross react with DNA from other human species of plasmodia except *P. knowlesi*. The htLAMP assay was then compared with nested PCR on a clinical sample set (n=42), showing a sensitivity of 100%, specificity of 48%, PPV 46% and NPV 100%. The assay turnaround time was 1 hour (following DNA extraction). In conclusion, this new *P. vivax* htLAMP assay shows promise as a sensitive, species-specific diagnostic tool for rapid detection of sub-patent *P. vivax* infections, and is amenable to epidemiologic and elimination activity in *vivax*-endemic settings.

835

PERSISTENT SUB-MICROSCOPIC *PLASMODIUM FALCIPARUM* WITH MUTANT CRT AND MDR1 GENOTYPES, A PROBLEM FOR MALARIA ELIMINATION IN THE PROVINCES OF SARANGANI AND TAWI-TAWI ON THE ISLANDS OF MINDANAO, PHILIPPINES

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The Philippines has set a national goal to eliminate malaria by 2020. In line with this, the country adopted artemether-lumefantrine (AL) as first-line treatment option for *Plasmodium falciparum* malaria in 2009. However, *P. falciparum* resistance to previously used antimalarials in the country such as chloroquine and amodiaquine might affect the usefulness of AL in the Philippines. In this study, the prevalence of *P. falciparum* was investigated in malaria endemic provinces of Sarangani and Tawi-Tawi in Mindanao, Philippines. *P. falciparum* was found in 1.8% (16/907) and 1.9% (21/1088) of cross-sectional survey participants by nested PCR using DNA extracted from dried blood spots on filter paper. These isolates were genotyped for previously reported polymorphisms in the chloroquine-resistance transporter (crt) gene and multi-drug resistance (mdr1) gene using real-time PCR, nested PCR and sequencing. At present, crt codons 72-76 have been analyzed in 14 and 21. *P. falciparum* isolates from Sarangani and Tawi-Tawi, respectively. Three of the 14 isolates from Sarangani (21.4%) harboured wild-type CVMNK, five isolates (35.7%) were CVIET, five (35.7%) were SVMNT, and one isolate was a mixed infection of CVMNK and CVIET. Four of the 21 isolates from Tawi-Tawi (19%) were CVMNK, one was CVIET, and 15 (71.4%) were SVMNT, with one mixed infection of CVMNK and CVIET. This was the first report for presence of *P. falciparum* with CVIET haplotype in Mindanao, Philippines. Both CVIET and SVMNT haplotypes have been previously associated with chloroquine and amodiaquine resistance. When the 16 *P. falciparum* isolates from Sarangani were genotyped for polymorphisms at mdr1 codons 86 and 184, 50% (8/16) have 86Y while 100% have 184F. Of the 11 out of 21 *P. falciparum* isolates from Tawi-Tawi genotyped to date for polymorphisms at mdr1 codons 86 and 184, 27.3% (3/11) have 86Y while 100% have 184F. These findings raised two issues relevant to malaria elimination in Sarangani and Tawi-Tawi: (1) the persistence of sub-microscopic *P. falciparum* circulating in human population despite existing control measures; and (2) the occurrence of *P. falciparum* with both CVIET or SVMNT haplotypes and mdr1 mutations are present in Mindanao.

836

USE OF PASSIVE AND ACTIVE SURVEILLANCE TO ASSESS THE ROLE OF HOUSING AS A POTENTIAL RISK FACTOR FOR LOCAL TRANSMISSION OF *PLASMODIUM FALCIPARUM* INFECTION IN SWAZILAND

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Understanding the role of housing as a potential risk factor for local transmission in Swaziland may highlight new areas for intervention as the country aims for malaria elimination. As part of a prospective study on active surveillance (investigation of index cases with screening their households and neighbors) we evaluated the relationship between housing and symptomatic or asymptomatic infection acquired locally. Symptomatic infection among index cases was diagnosed by *Plasmodium falciparum* Rapid Diagnostic Tests or microscopy and asymptomatic infection by Loop-mediated isothermal amplification (LAMP). The comparison group was LAMP-negative subjects screened in active case detection. Information about risk factors was collected through interviews and housing data collected by examination. To analyze relationships between infection and risk factors, logistic regression was performed. From Aug 2012 to Jan 2013, 197 malaria cases were reported in passive surveillance. Of 136 cases that were investigated, 30 were classified as locally acquired based on travel history. Of 779 household members and neighbors of index cases screened in active case detection, LAMP was completed in 477 and 8 infections were identified (1.68%). Compared to subjects living in a house with unscreened windows or no windows, mud, cane, grass or shrub internal or external walls, and a grass or palm roof, subjects living in a house with screened windows, cement block or brick external walls, cement block or brick or plaster internal walls, and a roof with metal sheets or tile had 5.2 higher odds of infection, 95% CI 1.6-17.1. Infection was also associated with reported travel within Swaziland, OR 2.42, 95% CI 1.0-5.8. There were no associations with age, sex, region, occupation, travel outside Swaziland, use of an insecticide treated bed net, or sleeping under a structure sprayed with insecticide in the past year. Adjusting for travel within Swaziland, higher quality housing had a 5.2 higher odds of infection, 95% CI 1.6-17.2. Unlike in other studies, poor quality housing was not found to be associated with infection. It is possible that in this low transmission setting, poor housing is not associated with infection. The association between higher quality housing and infection may be confounded by some other risk factor such as travel within Swaziland. Also, as our study is nascent and has not yet included wet season data, sample sizes for infected subjects were small.

837

MALARIA CASES AMONG MIGRANT WORKERS AT THE HYDRO-POWER PROJECT SITE IN BHUTAN

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There was a drastic decline in malaria cases in Bhutan with 12,591 cases and 22 deaths in 1999 to just 82 cases and one death in 2012. This decline has prompted program to shift from control strategies to elimination phase. Therefore, as the country moves into the elimination phase, monitoring the importation of cases is vital and imperative to prevent introduction of parasite into the areas where malaria vectors are prevalent and climatic conditions are suitable for transmission. Hence, this screening was carried out as a routing surveillance system to find malaria

parasites in migrant workers at two large hydropower projects in Bhutan. A total of 5797 migrant workers at two project site, Punatsangchu (4594 workers) and Mangdechu (1203 workers) were screened for plasmodium infections. Blood samples were examined using microscopy by the trained malaria microscopists over the period of 45 days and the positive cases were re-confirmed by rapid diagnostic test kits (RDT). There were eight positives cases with four cases *Plasmodium falciparum* and 4 cases *P. vivax*. Majority of workers were from West Bengal (27.77%). Most of the positive cases were from Jharkhand (4 cases). Among the positive cases only one patient had fever. Entomological surveillance found the presence of *Anopheles pseudowillmori*, *Aedes aegypti*, *Ae. albopictus* and sandfly in and around the project area. This finding indicates a high risk of malaria transmission and also other vector borne diseases as a result of parasite introduction from migrant workers. Since there are number of migrant workers from malaria ,dengue and kala-azar endemic states of India, there is a dire need to strengthen monitoring and surveillance of these workers to minimize disease transmission in the locality

838

ASSESSMENT OF INDOOR DENSITIES AND INFECTIOUSNESS OF MALARIA VECTORS IN THREE VILLAGES IN ULANGA DISTRICT, SOUTHERN TANZANIA

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High quality mosquito surveillance data is necessary to; assess spatial and temporal disease trends, evaluate new and existing interventions, identify transmission hotspots, identify dominant vectors and pathogens or detect new ones, to readily detect disease outbreaks and predict future disease trends. We established a longitudinal adult mosquito surveillance system in rural Tanzania, to provide essential data necessary for examining: 1) indoor densities of disease transmitting mosquitoes in the area, 2) prevalence of *Plasmodium* sporozoite among malaria vector populations in the area, and 3) the baseline malaria transmission intensity in the study area. From a population of 2433 households in 3 villages (Kivukoni, Minepa and Mavimba) in southern Tanzania, 1600 households were randomly selected and spatially assigned, based on latitudes, to 16 clusters each consisting of 100 households. Monthly mosquito collections were performed using CDC-Light traps inside 6 households randomly selected from each cluster. The mosquitoes were sorted by taxa and abdominal status, after which a sub-sample of the malaria vectors were examined by (PCR) to distinguish between sibling species. The vectors were also examined by (ELISA) to detect *Plasmodium* sporozoite in their salivary glands. Densities of the two main malaria vectors, *Anopheles gambiae* s.l and *An. funestus* were significantly associated with latitude (the cluster of sampling) ($df = 15$, $P<0.001$) and month of the year when the sampling was done ($df = 11$, $P<0.001$).The distribution of *Culex* mosquitoes was such that areas with the lowest densities of *Anopheles* were also the areas with the highest densities of *Culex*. There was also a clear temporal variation in densities of mosquitoes, the peak densities being observed around April and May. For *An. funestus* however, we also observed that the mean indoor catches tended to be higher during the dry season (starting May to August than the rest of the year.) The distribution of *An. gambiae* s.l was spatially clustered, mostly in a set of adjoining clusters centered on the middle of the study area while densities of *An. funestus* was higher in the southern part of the study area than in the rest of the area thus suggesting suitability of spatially targeted intervention.

839

COMMUNITY PERSPECTIVES AND PRACTICES RELATED TO OUTDOOR MALARIA TRANSMISSION IN RURAL TANZANIA

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Indoor malaria prevention methods such as Long-Lasting Insecticide Treated Nets have significantly reduced the disease burden in Africa, yet transmission persists in many communities, partly driven by mosquitoes that bite people outdoors. It is essential to consider perspectives of local communities towards this outdoor transmission, so as to inform the development of new tools for malaria control. We assessed views and behaviors of rural and peri-urban communities in southern Tanzania, regarding outdoor mosquito bites and malaria prevention. A cross-sectional survey was conducted in two rural and two peri-urban villages in southern Tanzania, using semi-structured interviews and structured observations. A total of 40 households were studied. The interviews assessed whether malaria vectors also bite outdoors and transmission can also occur outdoors, while the observations were used to identify common outdoor activities that expose people to mosquito bites and current means of protection against the outdoor bites. A prototype outdoor mosquito control device was then used to assess community responses towards such potential outdoor interventions in future malaria control. More than 90% of the respondents knew about malaria and had regularly experienced outdoor mosquito bites and complained of malaria persistence, but most of them still believed that transmission occurs mostly indoors such that some use repellents or long cloths to prevent themselves from biting. Common outdoor activities included shopping and socializing (30% of people observed), storytelling (21%), cooking (18%), eating (16%), fetching water (15%), all of which took place between 6pm and 11pm, and also starting 5am and 6:30 am, matching times when outdoor biting mosquitoes are also known to be most active. The respondents were willing to use and contribute towards financing of the outdoor devices for malaria control. In conclusion, the results show that people appreciate outdoor biting and the likelihood of outdoor transmission but use of intervention other than bed nets indoors, was rare. Providing well developed outdoor mosquito control devices that are acceptable will contribute substantially in reducing malaria transmission.

840

USING COMMUNITY KNOWLEDGE AND EXPERIENCES TO PREDICT DENSITIES AND DISTRIBUTION OF DISEASE-TRANSMITTING MOSQUITOES IN RURAL TANZANIA

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The current lack of reliable techniques that can be used for large scale programmatic monitoring of distribution and densities of disease transmitting mosquitoes is a major challenge to public health authorities, especially in low and middle income endemic countries. We describe here a new community-based participatory mapping approach that relies simply on the knowledge and experiences of residents to rapidly identify areas where disease transmitting mosquitoes are most abundant. The method is proposed for use in spatial targeting of mosquito control interventions. Such simplified methodologies for mapping outdoor vector densities will be particularly necessary for optimal placement of outdoor mosquito control devices, such as odour-baited mosquito traps or lure and kill stations. Step 1: Develop participatory representative maps of the study areas showing essential landmark features, which can be used by community members to classify mosquito densities. Step 2: Selected community members are asked to identify locations where they think mosquitoes are most abundant, by ranking the grids on a scale of 1-5. Step 3: The data generated is interpolated and classified to show places where people think there are high, medium of low mosquito densities. Step 4: Entomological sampling is conducted to verify outdoor mosquito densities in locations identified by community members as having high, medium and low mosquito densities. Step 5: Validation of the community based perceptions and data obtained from entomological sampling. Maps were derived from community knowledge and opinions on the mosquito density distributions. The maps were generated by interpolating the grid ranks as provided by the community members. The data is reclassified to show places identified having High, Medium and Low Mosquito densities. Interpolation was done using Inverse Distance Weighting method and Outdoor entomological sampling was conducted in areas that were identified by community members as having, High, Medium or Low

densities of Mosquitoes distributions. This study provides evidence that we can rely on community knowledge and experience to identify suitable areas where mosquitoes are most abundant and where to locate outdoor complementary interventions .Such method will be cheaper, quicker and easier and potentially will guide large scale implementation of outdoor control devices for lure and kill disease-transmitting mosquitoes.

841

TREATMENT OF ASYMPTOMATIC CARRIERS OF *PLASMODIUM FALCIPARUM* WITH ARTEMETHER-LUMEFANTRINE: IMPACT ON THE PREVALENCE OF ANEMIA

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The effect of systematic treatment of asymptomatic carriers of *Plasmodium falciparum* with artemether-lumefantrine (AL) on hemoglobin (Hb) levels and anemic status, compared with no treatment of asymptomatic carriers, was investigated in a 12-month, single-center, controlled, parallel, cluster-randomized study in 18 villages in Burkina Faso. Intervention and control village inhabitants participated in three community screening campaigns (CSC1-3) that took place approximately one month apart before the rainy season, and a fourth campaign (CSC4) after the rainy season had ended to mark the end of the study at12 months. The change in Hb level in all asymptomatic carriers aged >6 months from Day 1 to Day 28 of CSC1 was +0.53 g/dl (from 11.81 to 12.33 g/dl) in the intervention arm vs -0.21 g/dl (from 12.06 to 11.86 g/dl) in the control arm ($p<0.001$). During the same period, the proportion of asymptomatic carriers aged >6 months to <5 years with anemia (mild, moderate or severe) in the intervention arm decreased by 31.1% (from 75.7% to 44.6%), compared with a decrease of 4.7% (from 76.3% to 71.6%) in the control arm. After 12 months, the proportion of asymptomatic carriers with anemia was reduced in both arms. Systematic screening and treatment of asymptomatic carriers of *P. falciparum* with AL at the community level can reduce the prevalence of anemia in children in the short term (28 days), although the difference with the control arm was not maintained at 12 months.

842

QUANTIFYING MALARIA RE-INTRODUCTION RISK IN NAMIBIA IN A POST-ELIMINATION SETTING

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For the many countries pursuing malaria elimination, reintroduction risk from neighboring countries is a major concern. One of these countries is Namibia, which is nearing malaria elimination, but shares borders with countries with endemic malaria. A quantitative framework for predicting spatial risk of epidemic spread upon reintroduction could enable policymakers to more efficiently implement country-wide surveillance and control methods. We develop such a quantitative framework by combining spatial data on malaria transmission with a unique dataset on human movement between Namibian settlements. We hypothesize: 1) some settlements will be particularly susceptible to reintroduction, 2) some settlements will be epidemic sinks, or locations where epidemic spread is especially likely, and 3) these settlements can be predicted using a combination of transmission intensity and human movement metrics. We used an agent-based model (ABM) to simulate malaria transmission and assess likely paths of epidemic spread across 402 settlements in Namibia. Movement patterns were parameterized using a dataset consisting of mobile phone records for 1,191,000 people in Namibia, and potential transmission intensity was estimated using prevalence estimates for each settlement. Overall epidemic likelihood was low, due to low transmission rates country-wide. However, epidemic likelihood varied greatly dependent on the settlement where reintroduction occurred, ranging from 0% to 40% of reintroductions yielding an epidemic. In some cases, settlements close in proximity and similar in transmission intensity differed greatly in reintroduction risk, due to movement patterns of people in the settlements. Although urban areas have low malaria transmission, we found that certain commerce centers, such as Windhoek and Oshakati, were likely to receive malaria during an epidemic. These results help inform surveillance efforts in post-elimination strategic planning, and provide an understanding of the settlement characteristics involved in yielding and promoting spread of malaria epidemics.

843

ACTIVE SURVEILLANCE WITH REALAMP TO FIND ASYMPTOMATIC MALARIA INFECTIONS IN KANCHANABURI PROVINCE, THAILAND

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Early and accurate diagnosis of asymptomatic malaria infections is essential for malaria control programs, especially for countries focusing on elimination. Currently, active malaria surveillance in Thailand relies on microscopy or rapid diagnostic tests (RDTs); however, both methods have suboptimal sensitivity and specificity to detect low-density *parasitemia*s. As Thailand aims to eliminate malaria, improved methods for detecting submicroscopic malaria infections are needed. RealAmp integrates loop mediated isothermal amplification (LAMP) with fluorescence detection and can be done on a portable, battery operated device. A field study was undertaken in June 2012 to determine the utility of the RealAmp assay in detecting asymptomatic cases via active surveillance in Kanchanaburi province near the Thai-Myanmar border. Microscopy slides and dried blood spots were collected from 127 healthy individuals from 2 villages and a local school. Microscopy slides were examined by a microscopist at the local malaria clinic and an expert microscopist at the Bureau of Vector Borne Disease laboratory in Bangkok. Genomic DNA was extracted using 20% Chelex. All samples were tested in duplicate by real-time pooled PCR and RealAmp genus assay. A total of 7 positive samples (5 *P. vivax*, 1 *P. malariae*, 1 *P. falciparum* and *P. vivax* mixed infection) were identified by the reference pooled PCR method. The RealAmp assay correctly identified all 7 infections (sensitivity = 100% 95%CI: 59-100% and specificity = 97% 95% CI: 92-99%) while local and expert microscopy identified 3 and 4 infections respectively. The median age among those who tested positive was 22 years and 4 out of 7 were females. A majority (71%) of the positive cases were migrants living in Thailand and all but one reported agriculture as their primary occupation. Active surveillance using RealAmp detected more asymptomatic cases than either local or expert microscopy. These results have significant implications for local elimination efforts as migrant populations can introduce and facilitate transmission. RealAmp may provide an alternative to real-time pooled PCR as means to rapidly and efficiently detect asymptomatic malaria infections, which serve as important reservoirs.

844

DOES TYPHOID FEVER AWAKEN THE HYPNOZOITES OF *VIVAX* MALARIA?

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Persons with a single infection may develop another especially in the tropics during the pre-antibiotic era. When examining the old tropical medicine textbooks it was found that several authors (Laveran, Reed, Craig, James) noted the co-incidence of typhoid fever and *vivax* malaria relapse. Since fever has been postulated to trigger *vivax* relapses, we sought examples from the medical literature that might clarify if typhoid fever activates latent liver parasites (hypnozoites). Reed reported a series of 12 cases in US Army soldiers who had returned to the USA from Cuba in 1900 who had malaria relapses confirmed by microscopy following typhoid fever. Malaria relapses were noted to occur after becoming afebrile from typhoid. Simultaneous paratyphoid C and malaria epidemics were reported by Giglioli in an aluminum mining population in Guyana in 1926-27. Twenty-nine percent of paratyphoid patients also had *vivax* malaria on presentation and 32 / 82 cases (39%) died; one third of all deaths in hospital during the 1926 / 27 epidemic had both infections. During a 1928 typhoid epidemic in Fajardo, Puerto Rico there were 90 typhoid cases in a town of 15000. Of the 63 typhoid cases reviewed, 11 / 63 (17%) also had malaria parasites in their blood. During the Second World War in North Africa, 10 of 34 patients (29% of British soldiers, Italian Prisoners of War and Sudanese civilians) with typhoid fever also had *vivax* malaria. Typhoid fever can be associated with *vivax* relapses but proof of a causal relationship has not yet been found.

845

ASSESSMENT OF CLIENT SATISFACTION WITH INTEGRATED COMMUNITY MANAGEMENT PROGRAM: WAKISO, UGANDA

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Malaria, pneumonia and diarrhea are the leading causes of death in children under five in Uganda. In a bid to improve access to prompt effective treatment of these diseases, Malaria Consortium in partnership with United Nations International Children's Emergency Fund (UNICEF) introduced Integrated Community Case Management (ICCM) in eight central districts of Uganda. There is currently limited information on client satisfaction with this program. The main objective of this study was to assess client satisfaction with the ICCM program in Wakiso district, one of the implementing sites. A cross sectional survey using a modified SERQUAL tool was used to investigate client satisfaction with the ICCM program. The study population consisted of randomly selected care givers of children under the age of five years who had used ICCM services. Four hundred fifty four caregivers of children below five participated in the study. Data was collected using semi-structured interviews translated in Luganda after pre-testing the tool. Data was analyzed in STATA version10. Logistic regression was used to determine the association between client satisfaction and several factors. Among 454 respondents, 80% of the care givers of children under five were satisfied with ICCM program. The overall gap (-0.332) between expectations and perceptions was significant, (t=-4.89, p-value 0.0081) meaning that despite the high level of client satisfaction, there still exist a quality gap in services provided under ICCM. Furthermore, there were no significant differences in the expectation and perception scores among the different dimensions except for reliability which had a score of -0.49 (p-value 0.0005). The multivariable logistic regression model showed that primary education (OR 2.8, 95% CI 1.116-6.795) and being a Muslim (OR 2.9, 95% CI 1.403372-6.341) was significantly associated with client satisfaction. Overall, 80% of the clients were satisfied with ICCM services and there was no statistical significant difference between perceptions and expectations for all the dimensions except for reliability dimension. However, there is a quality gap in the services provided under ICCM services in Wakiso district. The DHO and implementing partners should study these gaps critically and develop relevant strategies to improve on the quality of ICCM services in regard to these variables.

846

RECALCULATING THE NET USE GAP: A MULTI-COUNTRY COMPARISON OF ITN USE VERSUS ITN ACCESS

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Use of insecticide treated nets (ITN) is widely recognized as one of the main interventions to prevent malaria and high use rates are a central goal of malaria programs. The difference between household ownership of at least one ITN and population use of ITN has in the past been seen as evidence for failure to achieve appropriate net use. We review data from the last seven years to recalculate the net use gap using the comparison indicator of 'access to nets within the household' as now recommended by RBM and WHO. Data from 36 DHS and MIS surveys (2005-2012) in sub Saharan Africa were used. For each dataset three indicators were calculated: access to and use of ITN by the population and household ownership of at least one ITN. Access was calculated by multiplying the number of ITNs in the household by 2 to obtain potential users in the household. This value was then divided by the de-facto members setting the value to 1 if potential users exceeded members. Use gap was expressed as the ratio between use and access. The range of values for household ownership of ITN was 3.5-90.9%, for access 1.5-74.5% and ITN use 0.3-68.4%. There was a close, linear relationship between access and ownership ($p<0.0001$, R-squared 0.93) showing the access result was on average 32% lower than ITN ownership. Overall the median proportion of users compared to those with access was 85.2% (Interquartile Range 71.5% to 99.6%) with 8 surveys (22%) showing proportions below 70% (range 11.2% to 69.4%) and another 8 surveys with a result above 100% (range 102% to 119%) indicating mean users per net exceeded 2.0 in these cases. Even at access levels <50% a median 81.2% used an ITN given they had access and this rate increased to 96.4% for access rates >50%. Linear regression of use against access showed an estimated use of 90.1% (95% CI 84.8-95.3) given access. However, the variation of use was high at lower access values and significantly decreased with increasing access (test for heteroskedasticity $p=0.008$) indicating more consistent use at higher access rates. These results clearly show that previous interpretations of low use rates as a failure of behavioral change communication to use nets was not justified and that low use rates were driven by lack of ownership instead. They also demonstrate the usefulness of the newly recommended distinction between use and actual access to ITN.

847

THE NATIONAL MALARIA PREVALENCE IN HAITI IS <1%

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Malaria is endemic in Haiti, although a recent prevalence estimate is not available. In December 2011, during the rainy season, a national, population-based Tracking Results Continuously survey was conducted, funded by Global Fund, to estimate malaria prevalence. A cross-sectional, two-stage cluster design was utilized where enumeration areas (EAs) were selected with a probability proportional to size and 20 households (HHs) within EAs were randomly selected. Questionnaires were conducted and all HH members were tested for malaria using three methods: rapid diagnostic tests (RDTs), microscopy by Haiti's reference laboratory, and polymerase chain reaction (PCR). RDTs were performed for 3944 persons,

16 (0.41%) were positive for *Plasmodium falciparum* (*Pf*) antigen and were treated. Of 3055 microscopy results, 12 (0.39%) were found with *Pf* parasites. Results from 2989 PCR tests showed 13 (0.43%) were positive. The number of persons positive for malaria by any single test was 27 and these were distributed among Haiti's ten Departments as follows: 11 (41%) Artibonite, 8 (30%) Ouest (3 in metropolitan Port au Prince); 3 (11%) Nord; 2 (7%) Nord Ouest, 2 (7%) Sud; and 1 (4%) Grand Anse. No other species of *Plasmodium* was found. The median age of the 27 test-positive persons was 6 years; range 1-51 years. Experiencing fever in the previous two weeks was reported for 18 (67%) persons; of whom 10 reported previous treatment with chloroquine. Nine of 27 (33%) had asymptomatic malaria, defined as the detection of *Pf* by any method and no reported fever in the previous two weeks. While the 3 testing methods produced similar estimates of malaria prevalence, there were discordant results between methods on the same samples. In order to monitor program progress over time, future surveys should include oversampling in areas suspected of higher transmission and using serology, a more sensitive test, to characterize malaria epidemiology in this low transmission setting. Malaria prevalence in Haiti is low and heterogeneous; these findings are encouraging for future elimination efforts.

848

EVALUATING THE IMPACT OF MALARIA INTERVENTIONS IN NIGERIA

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Malaria control has intensified globally with targets set by the World Health Assembly (WHA); the Roll Back Malaria (RBM) Partnership and the Millennium Development Goals (MDGs). These targets aim for the short-term goal of reducing the burden of malaria until it is no longer a public-health problem, and the long-term goal of reducing the global incidence to zero by progressively eliminating the disease in endemic countries.International funding for intervention scale up has risen steeply in the past decade. Goals are becoming more ambitious as countries record reductions in malaria incidence. In spite of this progress, Africa still bears the enormity of the global burden of the disease, with Nigeria accounting for a quarter of the disease burden in the continent. A review of evaluation studies on malaria interventions show that the link between intervention scale up and decreasing trends in malaria incidence and deaths in Nigeria remains unclear. This lack of clarity between intervention scale up and decreasing trends in malaria incidence underscores the need for this project, which seeks to evaluate the impact of scaled-up intervention coverage over the past decade in Nigeria. Data on prevalence, morbidity and mortality were extracted systematically from baseline evaluation studies of malaria transmission in Nigeria from the year 2000 onwards. These data were geo-located, classified bystate, and statistically analysed. Subsequently, intervention coverage data on mosquitonets, Artemisinin-based combination therapy rates and Intermittent Preventive Treatment were collated and evaluated. Descriptive analysis was undertaken on the intervention coverage data to characterise the scale up and to uncover spatial variation in interventions uptake. The results of these analyses informed the design of a survey to understand the impact of intervention scale up by region, and gaps between expected and observed impact. This survey focused on areas in which impact has been achieved and those in which further impact could be made to understand barriers to intervention effectiveness. Whilst the final results of this work is being aggregated, the main objective is to provide evidence-based recommendations to the NMCP regarding ways in which malaria control can be further improved in Nigeria, thereby offering further steps towards achieving the WHA, the RBM Partnership and the MDG targets for malaria in Nigeria.

849

ANTI MSP1 AND AMA-1 ANTIBODY DYNAMICS AND THEIR INFLUENCE ON THE OCCURRENCE OF SYMPTOMATIC EPISODES OF MALARIA IN A COHORT OF CHILDREN AGED BELOW FIVE YEARS

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MSP-1 and AMA -1 are key antigens that are produced during the asexual stages of the plasmodium life cycle. The relationship between the levels of antibodies to these antigens and their effects on control of *parasitemia* levels and protection from clinical disease is unclear. We studied the dynamics of these antibody levels and their influence on *parasitemia* levels and on the occurrence of symptomatic episodes of malaria in a subset of children from a cohort study that was conducted in Western Kenya between 2003 and 2004. A total of 270 asymptomatic children aged between 12 months and 47 months were randomized to receive either a course of Artemether Lumefantrine (Coartem) or placebo and then followed up for 1 year. Active surveillance consisted of weekly visits by field workers and monthly visits at the study clinic, during which laboratory assessments were carried out. Passive surveillance consisted of unscheduled visits. Immunological data was available for a subset of 60 children. The anti-AMA-1 and anti-MSP-1 antibody levels at baseline were higher in the older children. The youngest age category had an overall median anti-AMA-1 level of 2.8ug/ml compared to 31.8ug/ml and 100.5ug/ml in the older age categories. The overall anti-MSP-1 antibody levels showed a different trend, with the youngest age category having a median of 1.0ug/ml compared to 0.43ug/ml and 0.65ug/ml in the 24-35 month and 36-47 month age categories. The differences in median levels across age categories was statistically significant.There was a strong correlation between anti-AMA-1 antibody levels and *parasitemia* levels, and separately between anti-MSP and *parasitemia* levels. Lower antibody levels seen in subjects experiencing *parasitemia* levels ≥5000/ul as compared to those experiencing lower levels of *parasitemia*, and those with negative smears having the lowest antibody levels. Episodes of asymptomatic *parasitemia* were associated with higher anti-AMA-1 and anti-MSP-1 antibody levels in comparison with symptomatic malaria episodes and absence of parasites. This association was statistically significant. High anti-AMA-1 and anti MSP-1 antibody levels at baseline were not associated with a significant reduction in the number of clinical malaria. Similarly, the overall antibody levels were not associated with a significant reduction in the number of clinical malaria episodes.

850

DECLINING BURDEN OF MALARIA OVER TWO DECADES IN A RURAL COMMUNITY OF MUHEZA DISTRICT, NORTHEASTERN TANZANIA

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The recently reported declining burden of malaria in some Africa countries has been attributed to scaling-up of different interventions although in some areas, these changes started before implementation of major interventions. This study assessed the long term trends of malaria burden for 20 years (1992 - 2012) in Magoda and for 15 years in Mpapayu village of Muheza district, North-eastern Tanzania, in relation to different interventions as well as changing national malaria control policies. Repeated cross-sectional surveys recruited individuals aged 0 - 19 years from the 2 villages whereby blood smears were collected for detection of malaria parasites by microscopy. Prevalence and density of *Plasmodium falciparum* infections and other indices of malaria burden (prevalence of splenomegaly and gametocytaemia) were compared across the years and between the study villages. Major interventions and changes in

malaria control policies were also marked. In Magoda, the prevalence of *P. falciparum* infections initially decreased between 1992 and 1996 (from 83.5 to 62.0%), stabilized between 1996 and 1997, and further declined to 34.4% in 2004. A temporary increase between 2004 and 2008 was followed by a progressive decline to 7% in 2012, i.e. > 10-fold decrease since 1992. In Mpapayu (from 1998), the highest prevalence was 81.5% in 1999 and it decreased to 25% in 2004. After a slight increase in 2008, a steady decline followed, reaching <5% from 2011. Bed-net usage was high in both villages from 1999 to 2004 (≥88%) but it decreased between 2008 and 2012 (range, 28 to 68%). After adjusting for the effects of bed-nets, age, fever and year of study, the risk of *P. falciparum* infections decreased significantly, by ≥97% in both villages between 1999 and 2012 (p40% to <1%) and gametocytaemia (23% to <1%) also decreased markedly in both villages. In conclusion, a remarkable decline in the burden of malaria occurred between 1992 and 2012. The initial decline (1992 - 2004) was possibly due to the deployed interventions while the steady decline observed from 2008 (with bed-net coverage) suggests that other factors contributed to these changes. These results provide evidence that could be used to monitor progress towards elimination of malaria as a public health problem and reaching related MDGs.

851

COMPARISON OF AGE-SPECIFIC MALARIA MORTALITY RATES IN THE KEMRI/CDC HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN WESTERN KENYA, 2003-2010

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Malaria control interventions have been scaled-up in Kenya during the past decade. Most malaria interventions and burden assessments have focused on young children. Modeling of malaria burden in adults has produced highly variable estimates. To measure progress, accurate malaria burden estimates across age groups are necessary. We determined age-specific malaria mortality rates in western Kenya, an area of high malaria prevalence (38%, 2006), HIV prevalence (15% in adults, 2003-2004) and insecticide-treated net use (41%, 2006). We collected data from 140,000 persons in a health and demographic surveillance system from 2003-2010. Deaths were captured via community informant reporting and thrice yearly surveillance visits. Standardized verbal autopsies were conducted; probable cause of death was assigned by InterVA-4, a probabilistic method for verbal autopsy interpretation. Annual malaria mortality rates per 1,000 person-years (PY) were generated by age group. Trends from 2003-2010 were analyzed using Poisson regression. In children <5 years, the malaria-specific mortality rate (MMR) decreased from 13.2 to 3.7 per 1,000 PY, a mean reduction of 20% annually from 2003 to 2010. In children 5-14 years, MMR remained stable from 0.46 to 0.47 per 1,000 PY. In adults ≥15 years, MMR decreased from 1.5 to 0.42 per 1,000 PY for a mean reduction of 20% annually. From 2003 to 2010, the proportion of all malaria deaths by age group decreased in children <5 years from 69.8% to 61.4%, increased in children 5-14 years from 4.3% to 14.3% and decreased slightly in adults 25.9% to 24.3%. Malaria mortality rates in young children and adults have decreased dramatically from 2003 to 2010 in western Kenya, but older children have not benefited. Almost 40% of malaria deaths occur in older children and adults. Malaria deaths in adults might be overestimated due to HIV-associated mortality. Our data support current strategies to reach older children and adults and inclusion of all age groups in malaria control interventions, including universal coverage with insecticide-treated nets.

852

HOME INSPECTIONS TO VALIDATE CAREGIVER-REPORTED USE OF INSECTICIDE-TREATED BED NETS IN MACHINGA DISTRICT, MALAWI

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Studies evaluating use of insecticide-treated bed nets (ITNs) rely on self- or caregiver-reported use because direct observation at night is usually not possible. Such reports may be inflated, however, due to recall bias or social pressure to use ITNs, resulting in underestimates of true ITN effectiveness in reducing malaria risk. Following caregiver interviews conducted at centralized locations, we validated caregiver-reported ITN use through visual inspection of nets at the household. As part of a malaria cohort study in children (ages 6-59 months) in Machinga District, Malawi, caregivers were interviewed each month regarding their child's history of ITN use, including ITN use on the previous night. Between December 2012 and January 2013, a simple random sample of enrolled children (n=173) was selected and their caregivers were visited at their home within three days of the initial monthly interview by a surveyor who was blinded to their initial report of ITN use. The surveyor asked if the child used an ITN the previous night and, if the caregiver responded yes, requested to see the child's ITN hanging inside the house. The response from home inspections was scored as positive only if the child's ITN was visually observed. Due to problems with heavy rains and access to homes, median time from initial interview to home inspection was four days (range 0 to 14 days). Out of the 173 selected children, 30 had not slept in their own home the night before the initial monthly interview and were removed from analysis. For the remaining 143 children, 141 (98%) caregivers reported use of an ITN the night prior to the initial interview. Similarly, 141(98%) caregivers provided access to their child's ITN during the home inspection. However, 4 (2%) caregivers gave discordant responses in the home inspection compared to the initial interview. Considering the home inspection as the gold standard, the sensitivity of caregiver report was 98.6%. Due to the high level of ITN use, specificity could not be calculated. Results from caregiver reports and home inspections showed a high degree of agreement, indicating that ITN use patterns are high and consistent in Machinga District.

853

MALARIA IN PREGNANCY, LOW BIRTH WEIGHT AND PRETERM DELIVERY IN OUELESSEBOUGOU, MALI

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Pregnant women are at high risk of malaria infection that can lead to poor pregnancy outcomes including preterm delivery and low birth weight. To determine the frequency of malaria in pregnant women, and the risk factors for low birth weight and preterm delivery in Ouelessebougou, we enrolled pregnant women in a cohort study and followed them up to delivery. Blood smear was performed at enrollment, again between 30 and 32 weeks of gestation, on the day of delivery, and at the time of any

illness. Pearson's chi square test or Fisher's exact probability were used for comparison of proportions, and logistic regression was used for analysis of risk factors. 46.3 % (357/665) of pregnant women experienced malaria infection during pregnancy. The risk of pregnancy malaria (PM) was higher in primigravidae (OR= 2.41 [95% CI 1.62; 3.58]) and secundigravidae (OR= 1.74 [95% CI 1.15; 2.63]), compared to multigravidae. Use of ITN reduced the risk of PM by half (OR= 0.49 [95% CI 0.34; 0.70]). The proportion of low birth weight babies (LBW) was 9.6 % (61/636). Compared to multigravidae, the risk of LBW was higher in primigravidae (OR=3.84 [95% CI 1.98; 7.46]) and secundigravidae (OR= 2.39 [95% CI 1.17; 4.94]), adjusting for other factors such as use of intermittent preventive treatment with Sulfadoxine -Pyrimethamine (IPT -SP) and used of ITN. The frequency of preterm delivery was 10.4% (67/645). The risk of preterm delivery was higher for primigravidae (OR= 5.8 [95% CI 2.98; 7.46]), followed by secundigravidae (OR= 2.5 [95% CI 1.16; 5.28]). Use of IPT -SP was associated with reduced risk of preterm delivery (OR = 0.50 [95% CI 0.27; 0.84]). In conclusion, factors associated with malaria during pregnancy in Ouelessebougou were primiparity and use of ITN. Primiparity was also associated with the occurrence of low birth weight, while IPT-SP reduced the risk of preterm delivery.

854

MAPPING ARTEMISININ RESISTANCE: SMART SURVEILLANCE

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Artemisinin resistance has been detected in several *loci* in South East Asia. Current studies are ongoing to measure the extent of the spread. However, due to limited resources and the complexity of the methods used to characterize resistance, the number of sites remains limited. A methodology is proposed that will allow candidate sites to be chosen in an informed way and serve as a proof of concept for "Smart Surveillance" in general. Using malaria endemicity and human population density estimates in the Mekong region, along with maps of uncertainty in resistance based on resistance data, we aim to assess the effect of adding additional candidate sites to the dataset. The objective is to find the candidate sites that most reduce the uncertainty and maximize the useful information. Using parasite clearance half-life (HL) estimates from studies conducted in the Mekong region to characterize artemisinin resistance, the data are dichotomized in that we define the number of 'positive' responses as those with a HL above a cutoff. The cutoff is defined on a distribution of HLs in populations expected to be as sensitive to artemisinin as those observed in Africa. We develop a Bayesian geostatistical model of the proportion of individuals with a HL more than the cutoff. The model is fitted using a Markov chain Monte Carlo (MCMC) simulation and a predictive map is generated on a regular grid of the Mekong region, using the samples from the MCMC. For each prediction location, probabilities of the HL being greater than the cutoff are drawn and the distribution summarized as the median of this set. The associated uncertainty maps that accompany the median maps are created by calculating the coefficient of variation. The methods outlined here could play an important role in identifying where future efficacy studies should be done and inform decisions on surveillance of artemisinin resistance and elimination in the Mekong region.

855

CELL-MEDIATED IMMUNITY TO *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-1 IN KENYAN CHILDREN

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Merozoite surface protein-1 (MSP1) is an abundant, immunogenic blood-stage protein and a malaria vaccine candidate. In order to compare the development and maintenance of immunity to MSP1, we analyzed immune responses to T cell epitope regions from MSP1 allelic variant (3D7 and FVO) in 164 Kenyan children living in Kisumu County, a lowland region with holoendemic malaria, and Nandi County, a highland region with hypoendemic malaria. Samples collected in February 2003 and November 2004 were analyzed for *parasitemia* by microscopy, cell-mediated immunity by interferon-γ ELISPOT, and MSP1 genotype (3D7 vs. FVO) by polymerase chain reaction of the M33 fragment in block 16 of the gene. In 2003, 79% of Kisumu children and 12% of Nandi children were parasitemic; in 2004, 87% of Kisumu children and only 1 (1%) in Nandi were parasitemic. Based on sequencing of block 16 of MSP1, the 3D7 genotype was more common at both sites, detected in 48% and 73% of parasitemic samples in Kisumu and Nandi respectively. In Kisumu, ELISPOT positivity to 3D7 increased from 2003 to 2004 from 33% to 40% and magnitude increased by 72%; in Nandi, prevalence declined from 45% to 17% and magnitude declined by almost 75%. Odds of positive ELISPOT increased with age. ELISPOT reactivity was protective against concurrent *parasitemia* at both sites in 2003 (summary OR 0.45, 95% CI 0.22, 0.93) and in Kisumu in 2004 (OR 0.28, 95% CI 0.04, 1.50). In multivariate analyses, positive ELISPOT in 2004 was associated with older age, concurrent *parasitemia*, and past positive ELISPOT, though with the exception of age, confidence intervals included 1. These results suggest that cell-mediated immunity to MSP1 is protective against concurrent *parasitemia* but is short-lived, with immunity waning over time in the absence of continuous exposure to malaria.

856

AN INVESTIGATION OF MALARIA INTERVENTION IN MOZAMBIQUE THROUGH MATHEMATICAL MODELING

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Malaria has been a significant cause of morbidity and mortality in many Sub-Saharan African countries, especially in children under five years. A mathematical model was developed that could demonstrate malaria prevalence in Mozambique, and an intervention strategy was applied to the model to determine the effectiveness of the intervention. A modified Anderson-May compartmental model of the malaria disease-burden in Mozambique during 2008 was generated using differential equations in Berkeley-Madonna software by applying data obtained from census reports and literature review. The model split the human compartment into two groups, children under five years of age and all other ages, to reflect differences in malaria mortality related to lack of immunity in younger children and partial immunity in those over five-years of age. Intervention goals for insecticide treated net (ITN) usage as proposed by the President's Malaria Initiative (PMI) were applied to the model to test for effectiveness in reducing prevalence in the under-five population. Malaria prevalence and the basic (R_0) and net effective (R_n) reproductive numbers were measured. The model predicted the malaria prevalence among children under-five years to be 8% of the population, or approximately 1,790,000

cases; the prevalence among the over-five population was 24%, or 4,455,000 cases. R_0 in the older population was 9. R_0 in children under-five years was 1,142. After introducing 85% ITN usage, the prevalence in the under-five population had been reduced to 5.2%, or 1,163,916. The R_0 was reduced to 26 due to a decrease in mosquito bite rate. The R_n increased to 7.8 due to a higher number of susceptible in the population. The current intervention goal of 85% ITN usage temporarily reduced malaria prevalence by 2.9% in the children under five. This would delay onset of illness to an age when mortality from illness would be decreased. Multiple intervention methods could further reduce prevalence.

857

USE OF A TRIANGULATION APPROACH TO ASSESS URBAN MALARIA IN GHANA

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Public health decisions in Ghana have been informed by routine health service data suggesting that malaria is highly endemic throughout the country. A triangulation methodology was used to assess more rigorously the burden of malaria in the largest cities of the country. National stakeholders identified key questions which were addressed through analysis of existing data from household surveys (including the 2011 Multiple Indicator Cluster Survey - MICS), routine data from the Ghana Health Service (GHS), and previously published entomologic and epidemiologic research (including data accessed from the web site of the Malaria Atlas Project). Data were assigned to specific communities based upon geo-coordinates (where available) or the district in which they were collected. GHS data showed that 82% of all reported malaria cases and 95% of Accra cases were diagnosed without laboratory confirmation. Data from the MICS demonstrated that 80% of children in rural areas aged 5-59 months with a recent fever had a malaria infection (by rapid diagnostic test- RDT) but only 7% of febrile children from Accra were RDT positive. Published research as well as data from the MICS show that children in the largest cities of the three ecological zones were significantly less likely to be infected compared to children living in smaller communities of the same zone: 86% (95% CI: 66-94%) less likely in Accra vs. the rural coastal zone; 85% (95% CI: 66-93%) less likely in Kumasi vs. the rural forest zone; and 68% (95% CI: 37-84%) less likely in Tamale vs. the rural savannah zone. Laboratory confirmation of malaria is least common in Accra, where the prevalence of malaria is the lowest of any area of the country and where presumptive diagnosis is least reliable. This method, as yet little used in the malaria field, provides an evidence base for discussion among stakeholders and decision-making.

858

MOLECULAR EPIDEMIOLOGY OF PFCRT MUTANT HAPLOTYPES IN THE DEMOCRATIC REPUBLIC OF CONGO

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Mutations in the *Plasmodium falciparum* chloroquine resistant transporter (pfcrt) mediate resistance to multiple antimalarials including chloroquine (CQ) and amodiaquine (AQ). Despite the widespread prevalence of these mutations, chloroquine and amodiaquine remain possible alternatives to or partners with artemisinin drugs for treatment of *falciparum* malaria. Molecular surveillance for haplotypes conferring resistance to these drugs is necessary to understand the feasibility of the re-deployment of these drugs in practice. In order to describe the epidemiology of these genetic markers in the Democratic Republic of Congo (DRC), we genotyped parasites from 180 individuals sampled in the 2007 Demographic and Health Survey (DHS) using PCR amplification across codons 72-76 of pfcrt and direct sequencing. 166 (92.2%) samples were successfully genotyped. The wildtype haplotype CVMNK was present in 79 (47.6%), the chloroquine-resistant CVIET haplotype in 55 (33.1%), a mixture of CVMNK and CVIET in 31 (18.7%), and CVMDK in one (0.6%). Overall, 86 *parasitemia*s (51.8%) harbored the pfcrt K76T substitution which is highly correlated with *in vitro* and *in vivo* drug failure. The SVMNT haplotype, which is associated with reduced susceptibility to amodiaquine, was not observed. Geographic analysis of the distribution of parasites bearing CVMNK and CVIET haplotypes indicated that parasite populations are sympatric without clear geographic clustering. According to DHS data, chloroquine accounted for 19.4% and amodiaquine 15.3% of antimalarials administered for childhood fever; on a provincial level, there were no associations between prevalences of mutant haplotypes and the use of either drug. In the DRC, we document molecular evidence of substantial CQ resistance but no mutations associated with AQ resistance, suggesting contrasting durability of these antimalarials. This cross-sectional study of the genetic landscape of pfcrt in the DRC highlights the importance of continued surveillance of genetic markers of drug resistance to effectively inform public health policies.

859

IMPACT OF INCREASED DISTRICT-LEVEL INSECTICIDE-TREATED NET (ITN) DISTRIBUTION ON ALL-CAUSE UNDER-FIVE MORTALITY IN MALAWI, 2004-2010

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Insecticide treated mosquito nets (ITN) have been shown to be highly effective at reducing malaria morbidity and mortality in children. However, there are limited studies that assess the association between increasing ITN distribution and child mortality over time at the district level and under programmatic conditions. We used a Poisson model to assess the association between ITN program intensity and reductions in all-cause mortality in children less than five years of age in Malawi during 2004-2010 using district platform analysis. District-level ITN to population ratios were estimated using annual district ITN distribution data with a decay factor to account for loss of ITNs due to any cause (physical and/or chemical) in the previous years and mid-year population from census data. These ratios were then grouped into two categories representing low (<0.25) and high (0.25-1.2) ratios of ITNs available per person. Data from the 2006 Multiple Indicator Cluster Survey and the 2010 Demographic and Health Survey were used to construct annual district-level estimates for potential confounders; weighted averages were constructed for interim years. A multivariate generalized linear model with Poisson distribution controlling for household access to improved water sources, mother's educational level, prevalence of diarrhea, percentage of children under-five who were given vitamin A in the past six months, *Plasmodium falciparum* prevalence rate in children 2-10 years of age ($Pf PR_{2-10}$), and annual rainfall anomalies was constructed. In this model, higher district ITN program intensity was significantly associated with lower all-cause under-five mortality (incidence rate ratio = 0.85; 95% CI = 0.75-0.97). These findings suggest that increasing ITN distribution may have significantly contributed

to the decline in all-cause under-five mortality during 2004-2010 in Malawi and represent a novel use of district-level data from nationally-representative surveys.

860

FORECASTING MALARIA IN UGANDA WITH AGE AND ENVIRONMENTAL PREDICTORS

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Accurate predictions of malaria could provide public health and clinical health services with an opportunity to develop proactive, targeted approaches for malaria control and prevention that make effective use of limited resources. The objective of the research was to develop and evaluate the accuracy of malaria forecasting models for six different sentinel sites in Uganda, using clinical and environmental predictors. Health clinic data collected by the sentinel site surveillance program, Uganda Malaria Surveillance Project, was used in conjunction with satellite-derived rainfall, temperature, and vegetation estimates. Weekly, site-specific time series were created and examined for all variables. The potential lag between each predictor and confirmed malaria was incorporated into the Autoregressive Integrated Moving Average models. We generated a set of short-term, intermediate, and long-term forecasts of malaria prevalence at weekly intervals and the average forecast error was calculated, using a reserved portion of the training series. The temporal interaction of the environmental predictors and malaria ranged from one week to four months. The significance of rainfall, temperature, and vegetation as predictors differed across the sites, although generally, temperature and vegetation were better predictors of malaria when compared to rainfall. The forecasting accuracy improved with the series stratified by age group and the environmental predictors were significant for malaria prevalence for ages 0-5 and 5-15 but less often significant for ages 15 and over. The forecasting accuracy deteriorated with increased forecasting intervals and the short-term forecast accuracy ranged from 5% to 29% across the sites (mean absolute percent error). Our work demonstrates the utility of using environmental covariates for the prediction of malaria, when used in conjunction with historical counts of malaria stratified by age. Future work includes examining the predictive influence of treatment and malaria screening practices which we anticipate will improve the forecast accuracy of our models.

861

MALARIA EPIDEMIOLOGY IN FORESTED AREA OF CENTRAL VIETNAM: THE HIDDEN PARASITE RESERVOIR

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After successfully reducing the malaria burden to pre-elimination levels over the past two decades, Vietnam has recently engaged into a national malaria elimination program. However, in forested areas of Central Vietnam malaria elimination is likely to be jeopardized by the high occurrence of asymptomatic and sub-microscopic infections as shown by previous reports. We present the results of a malaria survey carried out in a remote forested area of Central Vietnam where malaria prevalence and risk factors were evaluated. After a full census (4 study villages=1,810 inhabitants), the study population was screened for malaria infections by standard microscopy and, if needed, treated according to national guidelines. An additional blood sample on filter paper was also taken in a random sample of the population for later polymerase chain reaction (PCR) and more accurate estimation of the actual burden of malaria infections. The risk factor analysis for malaria infections was done using survey multivariate logistic regression as well as the classification and regression tree method (CART). A total of 1,450 individuals were screened. Malaria prevalence by microscopy was 7.8% (ranging from 3.9 to 10.9% across villages): mostly *Plasmodium falciparum* (>81.4%) or P.*vivax* (17.7%) mono-infections; the large majority (69.9%) was asymptomatic. By PCR, the prevalence was estimated at 22.6%, (ranging from 16.4% to 42.5%) with a higher proportion of *P. vivax* mono-infections (43.2%). The proportion of sub-patent infections increased with increasing age and with decreasing prevalence across villages. The main risk factors were age (<30y), village, house structure, and no bed net. This study confirmed that in Central Vietnam a substantial part of the human malaria reservoir is hidden. Additional studies are urgently needed to assess the contribution of this hidden reservoir to the maintenance of malaria transmission. Such evidence will be crucial for guiding elimination strategies.

862

ASSESSING COST OPTIMIZED STRATEGIES FOR MAINTAINING AND EXTENDING THE GAINS AGAINST MALARIA

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Malaria burden has been substantially reduced in many endemic regions as a result of concerted efforts to scale-up insecticide-treated nets and effective treatment. Yet it remains unclear whether universal coverage of vector control will be required to maintain these gains, and whether permanent campaigns are feasible given the challenges of sustaining financing and preventing resistance. Alternatively, vector control may be withdrawn without risk of resurgence if sufficient fractions of malaria infections are cured, either through mass treatment or after being identified via case detection. Here, we describe a framework for defining the required surveillance infrastructure for a specific context that can identify and cure the fraction of infections required to maintain the gains or end transmission in the absence of continued vector control. Using the epidemiological contexts of Zambia and Haiti, we first estimate the intrinsic, spatially-varying risk of malaria by producing maps of R_0, the expected number of infections per infected human that would result in the absence of interventions. Second, we apply a mathematical transmission model to assess the fraction of infections that must be cured in order to maintain current prevalence or reduce it to zero. Third, we assess operational surveillance strategies that would be needed to achieve this threshold, including both passive and active measures. Fourth, we examine the relative costs of required surveillance strengthening compared to ongoing vector control. Initial results from Zambia suggest that prevalence can be maintained in areas where risk is very low ($R_0 < 3$) if active case detection supplements passive surveillance to identify and cure 30-60% of infections. In Haiti, strengthened passive detection combined with active measures, potentially including mass screening and/or treatment campaigns in transmission foci, will likely be sufficient to achieve elimination. Results suggest investment in surveillance-driven strategies may prove substantially cheaper and more sustainable than ongoing vector control.

863

ASSOCIATION BETWEEN INCREASING ITN USE AND REDUCTIONS MODERATE-TO-SEVERE ANEMIA IN CHILDREN 6 - 23 MONTHS OF AGE: A MULTI-COUNTRY DECOMPOSITION ANALYSIS

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Assessing the success of malaria control interventions is difficult due to the challenge of measuring malaria-specific outcomes. In contrast, hemoglobin levels are relatively simple to measure, and although not highly specific to malaria, moderate-to-severe anemia (hemoglobin < 8 g/dL) is a common symptom of the disease. Approximately 15% of anemia in pre-school-age children is attributable to malaria. To investigate the utility of moderate-to-severe anemia as an impact measure of malaria control interventions we analyzed data from 11 countries that had two or more demographic health surveys or malaria indicator surveys conducted between 2001 and 2011 containing information on insecticide-treated net (ITN) use and hemoglobin levels in children 6-23 months of age. Multivariate Oaxaca-Blinder decomposition for nonlinear response models with deviation contrast normalization for categorical variables were used to estimate the proportion of the decline in anemia prevalence over time due to increases in ITN use. Weighted average ITN use increased from 12.2% in baseline surveys to 44.3% in endline surveys and moderate-to-severe anemia decreased from 17.9% to 12.1%. In pooled, multi-country logistic regression models controlling for residence, household wealth, multiple birth status, mother's education, child's age and sex, and history of recent fever, odds of anemia were significantly lower for children who used an ITN the night before interview compared to those who did not (OR = 0.81, 95% CI = 0.70-0.94). Decomposition models reveal that the increase in ITN use between baseline and endline surveys accounted for 19% of the observed decrease in moderate-to-severe anemia which is equivalent to a 1.1% reduction in anemia prevalence. The changes in ITN use explained the greatest proportion of the total change in anemia between baseline and endline as compared to other covariates. Results suggest that scale-up of malaria control interventions are likely to have measurable impact on moderate-to-severe anemia and consequently that anemia may be a useful impact measure.

864

APPLICATION OF A *PLASMODIUM FALCIPARUM* WHOLE GENOME SNP MICROARRAY TO FIELD SAMPLES COLLECTED AS DRIED BLOOD SPOTS FROM SOUTHEAST ASIA

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Genomic epidemiology studies of *Plasmodium falciparum* provide insights into parasite population structure, gene flow, drug resistance and vaccine development. In areas with adequate cold chain facilities, large volumes of leukocyte-depleted patient blood may be frozen for use in parasite genomic analyses. In more remote endemic areas, dried blood spot samples can be collected and stored at room temperature. The small volume of blood preserved on dried blood spots limits DNA yield and may preclude whole-genome sequencing. Analysis of single nucleotide polymorphisms (SNPs) allows for rapid evaluation of genome-wide diversity. Here we describe a DNA microarray that was designed for use on low DNA field samples to type a genome-wide set of SNPs that prior sequencing had shown to be variable in Africa, Southeast Asia, and Papua New Guinea. Our microarray uses variable length cDNA probes to type 33,728 genomic *loci* in the *P. falciparum* genome. We measured SNP calling accuracy by hybridizing purified DNA from sequenced malaria lab strains, and consistently achieved >95% accuracy in these samples. We then tested field samples with significantly lower DNA concentrations extracted from dried blood spots collected in therapeutic efficacy studies and routine surveillance studies in Bangladesh, Cambodia, China, Myanmar, and Thailand. Our new high-density microarray provided high SNP call rates from a wide range of parasite DNA quantities (~2-250ng) determined by 18s qPCR extracted from whole blood and dried blood. Field samples with parasite DNA quantities below 2ng were whole-genome amplified before running on the microarray. The microarray assay performed adequately on dried blood spots, and may provide a useful tool for genome-wide analysis of malaria parasites in diverse settings.

865

THE BURDEN OF *GAMETOCYTEMIA* AND PLACENTAL MALARIA IN PREGNANT WOMEN IN BLANTYRE, MALAWI

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Women living in malaria-endemic areas, semi-immune to malaria normally, become newly susceptible to malaria infection during pregnancy. The goal of this study was to assess the extent to which pregnant women serve as a reservoir of malaria gametocytes, and thereby a source of continued transmission. We hypothesized that pregnant women may have significant *gametocytemia* due to chronic placental sequestration. We used specimens from an observational study of malaria during pregnancy conducted in Blantyre, Malawi in 2009-10. All women received sulfadoxine-pyrimethamine as intermittent preventive treatment and all episodes of *parasitemia* were treated. We performed light microscopy on all positive malaria smears to detect gametocytes. Placental samples were tested for active malaria infection by quantitative PCR and for past infection by histological examination for hemozoin pigment. Proportions were compared by Fisher's exact test. 2742 blood smears from 450 women were examined for evidence of malaria. Among these women, 79 (17.6%) were positive for asexual stages at least once, and among that subset, 11 (13.9%) were positive for gametocytes. In gametocyte-positive smears, the average gametocyte density was 70/µL blood. Placental specimens were available for 321 deliveries. *Gametocytemia* was strongly associated with active placental malaria (p=0.003), and past infection (p<0.001). All women with gametocytes detected in a peripheral blood smear had hemozoin detected in the placenta. The proportion of pregnant women with malaria infection who have microscopically detectable gametocytes is higher than is usually detected among adults in neighboring countries. Because malaria infection is frequent in the pregnant population and women are generally asymptomatic at the time of infection, pregnant women may serve as important reservoirs of transmission in malaria-endemic areas and this population may warrant specific attention in malaria elimination efforts.

866

AN UPDATE ON THE POTENTIAL FOR NORTH AMERICAN MOSQUITOES TO TRANSMIT RIFT VALLEY FEVER VIRUS

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Rift Valley fever virus (RVFV) poses a serious threat to both North America agriculture and to human health. As illustrated with the introduction and spread of West Nile virus, exotic pathogens have the potential to cause significant economic damage as well as illness in North America. Despite its potential to cause devastating injury to cattle, goats, and sheep, little is known about the potential for North American mosquitoes to transmit RVFV, and this information is needed to develop an appropriate response to the introduction of RVFV. Therefore, we have been evaluating the potential for a variety of North American mosquitoes to transmit this virus either horizontally or vertically. Data on the ability of >20 common North American mosquito species to become infected with and to transmit RVFV will be presented, including the ability of at least five species, representing two genera (*Psorophora* and *Mansonia*), for which there are no published data. While *Culex tarsalis*, *Aedes japonicus*, and *Psorophora ferox* were among the most competent laboratory vectors for RVFV, *Cx. quinquefasciatus*, *Cx. nigripalpus*, and *Anopheles crucians* were virtually incompetent, even when fed on hamsters with viremias $>10^{9.5}$ plaque-forming units (PFU)/ml. Although *Ps. columbiae* was among the most susceptible to oral infection, and most specimens tested developed a disseminated infection, there was a significant salivary gland barrier and none of the members of this species transmitted RVFV by bite, including those inoculated intrathoracically. In addition to laboratory vector competence, factors such as seasonal density, host feeding preference, longevity, and foraging behavior should be considered when determining the potential role that these species could play in RVFV transmission.

867

BLOOD-MEAL SOURCE INFLUENCES THE INTERACTION BETWEEN THE MALARIA VECTOR *ANOPHELES GAMBIAE* AND THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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Although it is widely accepted that *Anopheles gambiae* displays an extreme form of specialization for human hosts as a source of blood, it can also feed on a wide range of other vertebrate hosts. Recent studies have addressed the consequences of feeding on different vertebrate hosts on the fitness of mosquito vectors. However, it is currently unknown whether different blood-meal sources can also impact the development of malaria parasites within the mosquito vectors Here we explored the effects of a range of blood-meal sources on *An. gambiae-Plasmodium falciparum* interactions. Following an infectious blood-meal, mosquitoes were fed with blood from either chicken, cow, human, or sheep for the next 3 gonotrophic cycles. We then measured various fitness-related traits including mosquito fecundity, daily mortality rate and progeny development as well as parasite prevalence and intensity. Mosquito survival was strongly impacted by the blood-meal type, with avian blood substantially reducing mosquito survival. Compared to uninfected individuals, infected mosquitoes displayed reduced lifespan. Finally, we provide the first evidence that mosquito blood-meal source can modify the development of malaria parasites. Together, our data demonstrate that blood-meal source can shape *An. gambiae -P. falciparum* interactions and has ultimately the potential to influence the dynamic of malaria transmission.

868

EAVE CURTAINS: ENTOMOLOGICAL EVALUATION AND COMMUNITY KNOWLEDGE, ATTITUDES AND PERCEPTIONS IN PREVENTION OF MALARIA MOSQUITO ENTRY INTO HUMAN DWELLINGS IN KISIAN AND ROTA VILLAGES, KISUMU COUNTY, WESTERN KENYA

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Vector control has overwhelmingly relied on insecticides that target the adult stage of mosquitoes but with very scant consideration of simple changes in house designs that have potential of protecting people against malaria. Several studies have demonstrated that house modification with screens and ceilings reduces entry of mosquitoes into human dwellings, presumably reducing malaria transmissions. However community knowledge, attitudes and perceptions of such a tool remain unclear. Besides, community implementation of improved house designs is lacking. In addition, house modification may be countered with behavioral modifications from vectors that are exquisitely adapted to feeding and resting indoors. The purpose of this study will be to assess community knowledge, perceptions and attitudes towards house modification for vector control and to investigate the impact of eave screening on entomological indicators and changes in malaria care seeking behavior of individuals. The study will be conducted in Kisian and Rota villages in Kisumu county, western Kenya. One hundred and twenty pairs of neighboring houses with similar structural designs for each pair will be selected. For all the selected houses, a questionnaire assessing knowledge, attitude and perception of house screening for malaria control will be administered to household heads. In each pair, the houses will be randomly allocated to either receive untreated eave curtain or no eave curtain, controls. The intervention houses will be screened at the beginning of the study while control houses will be screened at the end of the study. Four rounds of sampling will be done fortnightly before screening and for three months thereafter by mechanical aspiration. The sampled mosquitoes will be identified both morphologically and by polymerase chain reaction (PCR), while female *Anopheles* will be tested for the presence of sporozoites and abdomens of fed and half gravid females will be analyzed for host blood meal detection. Results will be expected to show the community attitude towards house modification and possible behavioral modification by *Anopheles* mosquitoes due to changes in house design which will be critical for further vector control efforts.

869

DISENTANGLING THE IMPACTS OF SEASONALITY, TEMPERATURE AND LAND COVER ON THE ABUNDANCE OF ARBOVIRAL MOSQUITO VECTORS IN CALIFORNIA USING GENERALIZED ADDITIVE MODELS

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Mathematical models for the transmission of mosquito-borne diseases often rely on very simple assumptions about the population dynamics of the mosquito vectors. Linking such models to real-world trapping data on vector abundance requires a method that smooths over the discontinuous trapping data to yield complete time series, simultaneously accounting for the effects of other key environmental variables that also vary in space or time. Generalized additive models (GAMs) offer a flexible way to disentangle the relative roles of seasonality, temperature, and land cover as predictors of mosquito abundance, and here, we consider their ability to explain the spatio-temporal abundance patterns of the key arboviral vectors, *Culex tarsalis*, the *Cx. pipiens* complex, and *Aedes*

melanimon. Models were fitted to a very large surveillance data set from California (2003-2009, 102,188 trap-nights of 4,882,911 mosquitoes), and results will be used to inform models for the transmission dynamics of West Nile virus and Rift Valley fever virus. Preliminary results indicate significant effects of all three drivers (P<0.001), as well as significant interaction effects between day of year and temperature (P<0.001). The seasonal signal, as well as overall magnitude, for different land cover types is significantly different and matches known observed dynamics such as late-season flooding of wetlands that produces a late boost in abundance near wetlands. These model results provide a baseline for mosquito abundance that can be used in both persistence and invasion models of arboviruses, and the smooth functions that make up the GAMs allow for reasonable low levels of extrapolation. For example, since we account for both asynoptic temperature fluctuations and differentiate across land-types, we can both predict abundances in scenarios where there are small systematic increases (or decreases) in temperature throughout the year as well as scenarios where land types change through either conservation or urbanization.

870

THE IMPACT OF CLIMATE ON WEST NILE VIRUS TRANSMISSION ACROSS NORTH AMERICA

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In the fourteen years since West Nile virus arrived in North America, there has been enormous year to year variation. Three years, 2002, 2003, and 2012 had regional epidemics with more than 2500 neuroinvasive cases, 250 deaths, and many more febrile cases. In most other years there were relatively few cases. The causes of this year to year variation has not yet been identified, although climatic conditions have been invoked frequently. We present the results of an analysis of coarse continental county scale weekly data and detailed local scale to uncover the impact of climatic drivers. We find, somewhat surprisingly, that temperature and precipitation are not especially strong drivers of year to year variation, and the impact of temperature is nonlinear. These results highlight the importance of rigorous analyses of climate and vector borne disease, especially in the case of zoonotic pathogens where many factors can influence transmission to humans.

871

THE EFFECT OF DENGUE VIRUS INFECTION ON *AEDES AEGYPTI* RESPONSE TO DEET

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Mosquito behaviors, such as host-seeking, landing, probing and biting, are driven by chemical signals within the environment that are recognized by the olfactory system. Because dengue virus (DENV) replicates and remains at elevated levels within the mosquito brain during DENV infection, this pathophysiology may interrupt behavioral responses to chemicals used in products for protection from biting insects, potentially reducing intervention efficacy. Three different *Aedes aegypti* treatment groups, DENV-1 injected, diluent-injected, and un-injected, were exposed to DEET (2.5% and 0.14%) at various days post-injection (1, 4, 7, 10, and 14 dpi), in a laboratory assay designed to measure contact irritancy behavior in mosquitoes. Our results indicate a significant escape response in all test populations when exposed to DEET and this behavior did not differ significantly among DENV-1 injected, diluent-injected and un-injected groups. This outcome suggests that DENV-1 infection in mosquitoes does not interfere with the underlying mechanisms that drive an irritancy response to DEET, and that insect repellents may represent a viable tool for preventing contact between humans and DENV-infected mosquitoes. Ongoing studies include measuring Ae. *aegypti* gene-regulation after infection with DENV-1.

872

COMPARISON OF DENGUE VIRUS VECTOR COMPETENCE OF *AEDES MEDIOVITTATUS*, THE CARIBBEAN TREEHOLE MOSQUITO AND *AEDES AEGYPTI*

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Aedes mediovittatus is found throughout the Caribbean and often shares habitat with *Aedes aegypti*. After intensive vector control measures targeted the removal of *Ae. aegypti* in Cuba, it was reported that *Ae. mediovittatus* was able to invade and exploit these same artificial habitats. Vector control measures specific to *Ae. aegypti* in Puerto Rico may create a habitat vacancy that *Ae. mediovittatus* could exploit. A previous study showed that *Ae. mediovittatus,* as well as *Ae. aegypti,* can be infected by DENV-1 (PR-1318) and *Ae. mediovittatus* was more susceptible to infection with DENV-2 (New Guinea C, PR-1328) than *Ae. aegypti*. However DENV-3 and DENV-4 infection competence remains unknown. To determine the vector competence of *Ae. mediovittatus* for DENV 1-4, mosquitoes were infected *per os* with DENV-1 (HAW), DENV-2 (New Guinea C), DENV-3 (H87), DENV-4 (H241) at titers of 5-6 logs plaque-forming unit (pfu) equivalents. At 14 days post infection, samples were collected, RNA extracted, and tested by qRT-PCR to determine infection and transmission rates; results were standardized to pfu equivalents. A generalized linear model assuming a binomial distribution and using a log link was used to analyze infection rates. Infection rates between *Ae. aegypti* and *Ae. mediovittatus* are DENV-1 (15.0% vs. 13.3%), DENV-2 (16.7% vs. 25%), DENV-3 (10.0% vs. 18.3%) and DENV-4 (61.7% vs. 1.7%). Transmission rates between *Ae. aegypti* and *Ae. mediovittatus* are DENV-1 (6.7% vs. 11.7%), DENV-2 (1.7% vs. 1.7%), DENV-3 (5.0% vs. 6.7%) and DENV -4 (41.7% vs. 1.7%). Infection and transmission rates of the two species did not statistically differ for DENV-1, DENV-2, or DENV-3. *Ae. aegypti* had statistically higher infection and transmission rates for DENV-4. Vector control measures for dengue prevention in the Caribbean may need to account for *Ae. mediovittatus* which may be capable of transmitting DENV in the absence of *Ae. aegypti*. Future studies of *Ae. mediovittatus* will evaluate competence for recent dengue virus isolates.

873

MOSQUITO MAGNET® LIBERTY PLUS TRAP BAITED WITH OCTENOL CONFIRMED BEST CANDIDATE FOR *ANOPHELES* SURVEILLANCE AND PROVED USEFUL IN PREDICTING THE RISK OF MALARIA TRANSMISSION IN FRENCH GUIANA

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Malaria is a major public health problem in French Guiana. In the context to establish a policy to control and eliminate malaria, monitoring systems for anopheles populations that can contribute to predict epidemic risk and

to set priorities for malaria vector control are critical. Sampling *Anopheles* by the gold standard human landing catch (HLC) method faces ethical constraints. Alternative traps are available but their efficacy varies and depends on mosquito behaviors. *Anopheles darlingi*, the main vector in French Guiana, bites and rest outdoors in inland Amerindian villages. We first evaluated the efficacy of Mosquito Magnet® trap baited with Lurex3™ (MM1) or octenol (MM2) and double mosquito net (DMN) baited with human against HLC for sampling outdoor anopheles. Association between anopheles densities collected with the most efficient trap and HLC was then investigated in a year study. Finally, the selected trap was used to collect entomological data and analyzed for association with clinical malaria cases after a six-month study aiming at predicting the risk of contracting malaria. In all, 361 anopheles were collected during traps evaluation and only sampling by MM2 showed no statistically difference with HLC (p=0.1178). Of the 325 *An. darlingi* captured, MM1 contributed 9%, MM2 29% and HLC 62%. Under the year long study, MM2 and HLC showed strong association (p<0.001, Spearman's coefficient: 0.7280) when analyzed for An. *darlingi* collections (n= 5726). Under the six-month study, 216 An. *darlingi* were collected with MM2 and 136 malaria cases were reported by the local health center. A significant correlation (p=0.0003; Spearman's r = 0.6494) between An. *darlingi* density and clinical malaria cases was noted. MM2 demonstrated potentials as a replacement for HLC in providing useful information needed for *Anopheles* surveillance in view to implement an efficient and sustainable malaria control strategy. Further assessment of MM2 for anopheles collections in French Guiana and modeling its data for predicting human biting rate is recommended.

874

ENTEROBACTER SP. IN THE MOSQUITO GUT: LPS DEFICIENCY AFFECTS GUT COLONIZATION AND MALARIA SUSCEPTIBILITY IN *ANOPHELES GAMBIAE*

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The mosquito gut ecosystem harbors a complex microbial community, which varies across life stages. Mosquitoes take blood meals for egg production. Blood feeding enables disease transmission. Blood meals can significantly reduce the community diversity and favor enteric bacteria. *Enterobacter sp.* is a dominant bacterium that expands after blood feeding in the gut of the mosquito *Anopheles gambiae*. Therefore Enterobacter could be a good model bacterium to study bacterial behavior in the blood fed gut. We isolated a strain of *Enterobacter sp.* and sequenced its genome. To be able to study genes that are involved in the colonization of the gut, we conducted mutagenesis in *Enterobacter* and generated a mutant library using EZ-Tn5™ Tnp Transposome Kit (Epicentre). Three mutants were identified, in which alpha1,2-glucosyltransferase (waaJ), O-antigen ligase (waaL), LPS heptosyl transferase I (waaC), were disrupted by insertion, respectively. These genes are clustered in the same genomic region, and all involved in LPS biosynthesis. These mutants have deficient LPS structure, as shown in the PAGE gel. In addition, the mutants are much more sensitive to paraquat, an strong oxidative stress inducer, compared to the wild type. We tagged wild type and waaL mutant with GFP or RFP, respectively. Tagged bacteria were traceable after introducing into the gut via oral feeding. Tagged wild type (wt) bacteria well proliferated after blood feeding, and excreted in the feces around 48hr post feeding. However, the capacity of gut colonization was compromised in the LPS deficient mutants. In addition, the LPS deficiency also affected mosquito susceptibility to malaria. When infected with malaria *Plasmodium berghei* the mosquitoes with wt bacterial reconstruction and unmanipulated control mosquitoes had similar infection pattern, while mosquitoes with mutants had significant higher oocyst load. The data suggest that bacterial LPS structure is important for the gut colonization. The investigation of the effects of LPS deficient mutants on the gut basal immunity is under way.

875

TRANSMISSION OF DENGUE HEMORRHAGIC FEVER IN ITS RELATIONSHIP BY CLIMATE VARIABILITY IN JAKARTA, INDONESIA

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Dengue hemorrhagic fever (DHF) has become endemic in many big cities in Indonesia. The Indonesian geography, the population growth, the climate change, the low level awareness and knowledge have caused the DHF taking place, which more over tending to increase in number. The objective of this research is to identify the transmission dynamic of DHF cases related to the pattern of the climate variability in Jakarta. The study was conducted in 5 sub district from April 2012 until March 2013. This research uses the design of ecological study with hypothesis test model and statistical analysis. Respondents of 844 households were interviewed to explore their knowledge, attitude and practice (KAP) regarding DHF using a standard questionnaire. The result indicate, that the DHF cases in Jakarta are influenced by precipitation (0.000), temperature ambient (0.000), indoor humidity (0.003), outdoor humidity (0.000), Man Landing Rate *Aedes* (0.016), resting habit *Aedes* (0.000) and community knowledge (0.008). The most influential climate factors to the DHF cases are precipitation, temperature, humidity and the low level of the community knowledge. The simulation model indicates a factor that may decrease DHF was mosquito larva monitoring and knowledge enhanced.

876

ESTIMATING THE HERITABILITY OF EXTRINSIC INCUBATION PERIOD IN DENGUE INFECTED MOSQUITOES

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In the past 20 years, dengue has re-emerged to become the most prevalent arthropod-borne virus affecting humans today. This exponential increase in disease incidence has brought with it significant health, social and economic problems. Dengue is most commonly vectored by the mosquito *Aedes aegypti*. Vectorial capacity, which is a measurement of the efficiency of vector-borne disease transmission, is influenced by a few key factors. Extrinsic incubation period (EIP), which is the interval of time from the ingestation of an infectious bloodmeal to the time of transmission, is a key determinant of vectorial capacity. Previous studies have estimated the heritability of susceptibility of *Ae. aegypti* to dengue and used quantitative genetic techniques to identify *loci* that underpin this trait. The genetic basis of EIP in mosquitoes is not well understood largely due to the technical difficulty in measuring this trait. Here we have carried out a quantitative genetic study using a full-sib breeding design to estimate the heritability of EIP in dengue infected *Ae. aegypti*. Using a non-destructive method to measure EIP we have also been able estimate heritability of body size, susceptibility to dengue infection and viral titer in the same mosquitoes. The heritabilities and correlations between these four traits reveal the genetic architecture of vector competence and the evolutionary potential for EIP.

877

MOSQUITO SALIVA FACILITATES CHIKUNGUNYA VIRUS INFECTION AND DISSEMINATION

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Chikungunya virus (CHIKV) is an alphavirus belonging to the Togaviridae family that is transmitted to humans by infected mosquitoes. The virus is delivered to humans together with mosquito saliva at the bite site. The complex repertoire of secreted bioactive molecules in mosquito saliva can have profound effects on transmission efficiency, pathogen establishment, and disease pathogenesis. In this work, we have investigated the role of mosquito saliva to facilitate CHIKV transmission and establishment of infection. Three groups of mice were intra-dermally inoculated with a) Salivary gland extracts (SGE), b)CHIKV+SGE, c) CHIKV alone. were intra-dermally delivered to mice ear with and without CHIKV. Skin biopsy at the site of infection (ear pinna), blood, tissues (auricular lymph nodes, spleen, liver, brain, skeletal muscle), and limbs were collected at 48, 72 and 96 hours post-infection (hpi) for comparative histological and molecular analysis. In skin, IL-1β, IL-6, IFN-γ, CCR1, CCL7, CCL2 and TNF-α were significantly upregulated in CHIKV+SGE injected animals compared with that of CHIKV alone. This suggest a potent innate-like immune response dominated by macrophages and neutrophils. In the lymph nodes, IL-12, IFN-γ, CCL7, CCL-2, CXCL5 were significantly upregulated in CHIKV+SGE injected animals compared with that of CHIKV alone. In muscle, IL-12, IFN-γ, TNF-α, CXCL5, CCL7, CCR1 were upregulated. In the early time points of this study, a mixed immune response was observed. Both IL-12 and IFN-γ were significantly upregulated after 72 hpi suggesting a Th1 type immune response at the later stage. Overall, pro-inflammatory cytokines/chemokines from the skin, lymph node and muscle were upregulated. CHIKV was detected in skin, muscle, lymph node and spleen samples, but not in brain and liver samples. Inclusion of SGE with CHIKV resulted in enhanced/elevated viremia in skin, lymph nodes and muscle of animals compared to that of CHIKV alone. These data clearly show that Ae. *aegypti* SGE enhances the key process of early CHIKV infection and dissemination to lymph nodes and muscle.

878

VECTOR COMPETENCE OF *CULEX PIPIENS QUINQUEFASCIATUS* (*DIPTERA: CULICIDAE*) FOR WEST NILE VIRUS ISOLATES FROM FLORIDA

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Culex pipiens quinquefasciatus is a vector of West Nile virus (WNV) in the United States. Four WNV isolates (WN-FL-03, WN-FL-05-558, WN-FL-05-2186, and WN-FL-05-510) collected from different regions of Florida (FL) were used for vector competence experiments in FL *Cx. p. quinquefasciatus.* Vector competence was compared between two mosquito colonies for WN-FL-03. Mosquitoes were fed blood containing 7.9±0.2 $\log_{10}$ plaque-forming units WNV/mL ± SE and incubated at 28°C for 14d. Vector competence was evaluated (χ^2,P<0.05) for rates of infection, dissemination, and transmission and virus titer in bodies, legs, and saliva was compared (ANOVA, P<0.05). Infection and dissemination rates (≥ 95%) were not affected by isolate or colony. Transmission of WNV (0-20%) was affected by isolate and colony and was observed only in mosquitoes fed WN-FL-05-558 and WN-FL-05-2186. Significant differences were observed between isolates and colonies for WNV titers of body and leg tissues, but not saliva. The highest body titers were observed in mosquitoes fed WN-FL-05-558 and the highest leg titers were observed in those fed WN-FL-05-558 or WN-FL-05-2186. High leg titers in the group fed WN-FL-05-558 or WN-FL-05-2186 were associated with transmission. *In vitro* growth rate in Vero cells was evaluated for WNV isolates. Supernatant was sampled at 24h, 48h, 72h, 96h, 120h, 144h, and 168h post-inoculation. Viral titers in WN-FL-03 were significantly higher (ANOVA, P<0.05) than other isolates from 24h–144h except for WN-FL-05-558 that was highest at 120h. No titer differences were observed between isolates at 168h. Variation in vector competence and *in vitro* growth rates between isolates may be related to genetic differences shown by a previous study. Low transmission rates suggest that isolates were affected differently by the salivary gland transmission barrier. Geographic variation in vector competence of mosquitoes may be attributed, in part, to their exposures to WNV isolates with different growth rates. The evaluation of differences in vector competence and *in vitro* virus growth kinetics for WNV isolates from different regions may help us understand vector-virus interactions and, hence, the role of vectors in virus transmission cycles in nature.

879

AEDES ALBOPICTUS AS MAIN VECTOR OF FIRST DENGUE HEMORRHAGIC FEVER OUTBREAK IN KAIMANA DISTRICT WEST PAPUA: AN ENTOMOLOGY SURVEY AND MOLECULAR APPROACH

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Kaimana District was an coastal area in West Papua (0-100 above sea level). Dengue outbreak first case 50 years had been occured in May-July, presumptively caused by transmission of Dengue virus from infected temporer visitor that participating one of religious event, held by local goverment. Entomological survey was intended to identified the species, breeding habitat of suspected mosquito vectors and detection of the present of virus in mosquitoes as well as in larvae. Investigation were overtaken on Juny 2012. Mosquitoes sample were collected on resting habitat as well as human landing collection. Larvae were collected from water container at 25 sampling site consist of patient household, school, and hospital. Larvae collection was rearranged in Entomology Laboratory. Dengue virus were detected in mosquitoes that had been collected from research area as well as from rearranged mosquitoes using RT-PCR method Lancioti primer. Case report show that the age majority of DHF cases is 6-12 (48,1%) and dominan patients were woman (63%). The study found one dead (3,7%) cases and 26 (96,3%) sick cases. Result of entomology survey indicated that *A.agyepti* had been known as dengue main vector in most area in Indonesia were absent, while the second vector *A.albopictus* was present abundantly in adult stage as well as in larvae stage that had been collected. The mosquitoes were found more in their resting habitat than in patient's houses. The most abundant of the mosquitoes was found at 13.00 in SDN Kaimana one of MTQ event took place. The container that contain *A.albopictus* larvae were unused tire, plastic container and ceramic container. The study from 25 sampling location indicated *House Indek* 26,6% and *Container Indek* 21,2% . The house index was more than 10% mean that this area has high susceptibility in DHF outbreak according to Ministry of Health criteria. Molecular detection of virus on samples showed that the dens virus was been detected on mosquitoes as well as on larvae.

880

MALARIA RISK AMONG FISHERMEN AND SUBSISTENCE FARMERS OF RUSINGA ISLAND, WESTERN KENYA

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Malaria is a leading cause of morbidity and mortality in Kenya. The highest burden of malaria occurs in the western part of the country where transmission occurs throughout the year. Evidence suggests that malaria transmission is unevenly distributed between individuals in populations. Some people are at a higher risk of infection than others. Although

malaria transmission is higher in western Kenya than other areas of the country, there are factors that influence the risk of transmission, such as peoples' behavior patterns, environmental factors, and socio-economic status. A cross-sectional study was conducted in western Kenya to determine factors linked to livelihood-related activities that influence malaria risk. A total of 248 fishermen and 114 farmers were randomly recruited into the study. House to house visits were conducted whereby participants were interviewed using a structured questionnaire and blood test for malaria infection administered. The variables that were studied included location of residence (zone), mode of dress while carrying out livelihood activities, housing characteristics, time of work, and use of malaria protection measures while working. The relationship between variables and malaria were analyzed by fitting a generalized linear model (GLM). Fishermen and farmers living in two zones were six times more likely to be infected with malaria than their counterparts in the other six zones. Malaria infection was significantly higher in individuals who dressed scantily (clothes that exposed their arms and legs) while working outdoors than among counterparts who were fully dressed. Individuals who worked at night were more likely to suffer from malaria than those who worked during the day. Fishermen and farmers both work at odd hours during peak biting times of malaria-transmitting mosquitoes, that is, during twilight zones and at night. In conclusion, location of residence, mode of dressing while at work and time of work were prominent risk factors for malaria infection among fishermen and farmers of Rusinga Island.

881

AUTODESSIMINATION OF PYRIPROXYFEN TO *ANOPHELES ARABIENSIS* BREEDING HABITATS: A NOVEL LARVICIDING APPROACH

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While conventional larviciding could greatly complement current malaria vector control interventions, its high operational cost and the difficulty in locating all mosquito-breeding habitats in rural settings reduces its effectiveness. Autodissemination of larvicides by mosquitoes offers a new possibility for more effective larviciding. This study assessed the potential for *Anopheles arabiensis* to transfer pyriproxyfen (PPF), a potent larvicide, from contamination sites to their breeding habitats. Different mechanisms for contaminating An. *arabiensis* were tested inside separate sections of a semi field system (SFS) in rural Tanzania. We assessed whether a cow, clay pots, or cow shed roofs and walls sprinkled with PPF powder, could contaminate mosquitoes while blood feeding or resting. Treatment was done using 10% AI PPF powder, and a control was left untreated. In each SFS section, a cow shelter (mud hut) was built, clay pots were provided as resting sites and artificial breeding habitats were created. Unfed adult female An. *arabiensis* were released inside the SFS and a cow was provided for blood meal. Egg and larval presence were monitored daily from the breeding habitats provided, and pupae moved into cages to allow for exact emergence rates to be calculated. Using a treated cow, mosquitoes were contaminated with PPF during blood feeding and larval bioassays using these mosquitoes demonstrated a significant adult emergence inhibition, proving that mosquitoes were able to pick up PPF. With clay pots, mosquitoes were contaminated while resting and a 90% adult emergence inhibition was observed in breeding habitats in the same section, suggesting that PPF had been disseminated by the contaminated mosquitoes. Similarly, treating the cow shed successfully contaminated resting mosquitoes and completely prevented them from laying eggs. These findings indicate the possibility of auto-dissemination of PPF by *An. arabiensis* but also the sterilization of adult mosquitoes with this insecticide.

882

THE ROLE OF CD8+ T CELLS IN PATHOLOGICAL GIARDIASIS

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Infection with the protozoan *Giardia duodenalis* is the most common intestinal parasitic disease in the United States. Disease transmission occurs with consumption of cyst-contaminated food or water. Clinical giardiasis presents with diarrhea and malabsorption of nutrients. Animal models have been helpful in defining the molecular processes that impair nutrient absorption and induce osmotic shifts within the infected gut to cause diarrheal disease. One major pathological hallmark of *Giardia* infection is the reduction of intestinal digestive enzymes, such as disaccharidases. β2m-/- mice, that lack functional CD8+ T cells, do not exhibit reduced disaccharidases and yet clear infection with normal kinetics. Disaccharidase reduction has also been recapitulated in athymic mice receiving CD8+ T cells from infected donor mice by adoptive transfer. This suggests that CD8+ T cell responses during *Giardia* infection are strictly pathogenic. In light of these observations, we have devoted our efforts to understanding how G. duodenalis infection activates intestinal CD8+ T cells to drive pathology. We report an increase of CD8+ T cells within the duodenum of infected mice. Analysis of surface marker expression on intestinal CD8+ T cells revealed an increased CD44hi population following infection within the lamina propria. These CD44hi cells produced IFNγ and TNFα. *Giardia* is non-invasive and persists within the intestinal mucosa without breaching the epithelial barrier or infecting host cells directly. Therefore, it is interesting that a CD8+ T cell response is triggered and that this response does not confer protection but rather leads to intestinal injury. The dependence on antigen in this response is currently being pursued in order to determine if *Giardia*-activated CD8+ T cells are mounting a specific response or are acting as bystanders. Further characterization of these intestinal CD8+ T cell responses to *G. duodenalis* infection will expand our understanding of pathogenesis and help identify novel therapeutic targets for the clinical setting.

883

PRIMARY AMEBIC MENINGOENCEPHALITIS ASSOCIATED WITH THE PRACTICE OF RITUAL NASAL RINSING - ST. THOMAS, U.S. VIRGIN ISLANDS, 2012

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Primary amebic meningoencephalitis (PAM), an almost universally fatal condition, affects 0-8 persons annually in the U.S. PAM results when *Naegleria fowleri*, a free-living ameba found in warm freshwater, enters the nose and migrates to the brain. While most infections are associated with recreational freshwater exposure, nasal rinsing has recently emerged as a mode of transmission. On November 21, 2012, the U.S. Virgin Islands (USVI) Department of Health documented the first PAM infection and death in the territory. Infection occurred in a 47-year-old Muslim male who practiced ablution, a required ritual cleansing done in preparation for prayer that sometimes involves nasal rinsing. An investigation was conducted to characterize the patient's water exposures and describe community ablution practices. In December 2012, semi-structured interviews were conducted with the patient's roommate and a snowball sample of participating members of his mosque. Environmental investigations, including water sampling, were conducted concurrently at the case-patient's home and mosque. The patient had no recreational freshwater exposure and primarily practiced ablution at home using untreated rainwater and groundwater and at the mosque using municipal water. All 22 participants practiced ritual ablution; 86% performed nasal rinsing. To be acceptable for ablution, water must simply look, smell,

and taste clean. Municipal water and rainwater were commonly cited as ablution water sources (86%). Groundwater and lake water, were also considered acceptable (59%). In total, 18% (3/17) of samples from the patient's home, including one sample from the water heater, yielded *N. fowleri*; none of the three samples from the mosque yielded *N. fowleri*. Although the patient was likely exposed to *N. fowleri* at home, his primary water source mirrors those of other mosque members, demonstrating an ongoing risk for PAM in the USVI Muslim community. Culturally appropriate education materials on water treatment and PAM are needed.

884

IDENTIFICATION OF A CANDIDATE SEROTONERGIC RECEPTOR IN *ENTAMOEBA INVADENS*

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Entamoeba histolytica, the etiologic agent of amebiasis, is one of the leading causes of parasitic disease, especially in resource limited countries and regions with poor sanitation. The life cycle of Entamoeba alternates between a feeding trophozoite and dormant cyst stage, the latter of which is passed from host to host, therein transmitting infection. The mechanism by which the parasite encysts, or transforms from the trophozoite to cyst form, is not well understood. Previous research has suggested the involvement of classical adrenergic, serotonergic, and histaminergic signaling pathways via G-protein coupled receptors (GPCR) and a cyclic AMP cascade. Agonists of these respective pathways were found to stimulate encystment while antagonists prevented encystment. The object of this study is to verify the presence of a potential serotonergic receptor and understand its role in the process of encystment when bound to serotonin-like agonists. Using the related species *E. invadens* as a model, the likely genomic sequence coding for the GPCR has been identified based on sequence alignment with known eukaryotic GPCRs. The gene has been PCR amplified and cloned into a pET102/D-TOPO® *E. coli* vector for bacterial expression and *in vitro* transcription/translation system expression. Expression of the protein in each system has been verified by western blotting. We will now use radiolabeled serotonin-like ligands to confirm the GPCR-ligand relationship, screen libraries of small molecules for novel antagonists, and determine the structure of the ligand binding site for future design of amoeba-specific inhibitors.

885

TOXOPLASMA GONDII: A REVIEW OF THE RELATIONSHIP BETWEEN SEROPOSITIVITY AND PSYCHIATRIC MORBIDITY

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Toxoplasma gondii is an obligate protozoan parasite that has the ability to invade and infect all mammalian cells, except erythrocytes. Domestic cats are the definitive host, acquiring *T. gondii* by ingesting prey such as birds and rodents that are infected . The parasite may exist in three forms: oocyst, tachyzoite and bradyzoite. Human infection occurs from ingestion of contaminated food or water, unprotected exposure to litter boxes of acutely infected cats, and congenital transmission. *T. gondii* has been demonstrated to reside in neural and brain cells, based upon post mortem analyses that showed involvement of both hemispheres. Given the ability to invade and infect brain tissue, researchers have explored the possibility of a link between *T. gondii* infection and psychiatric disorders, particularly schizophrenia and major depression. The purpose of this review is to evaluate the current literature regarding a possible causal link between *T. gondii* infection and such psychiatric disorders.

886

DETECTION AND GENOTYPING OF *TOXOPLASMA GONDII* IN HIV AND BLOOD DONORS AT THE KORLE-BU TEACHING HOSPITAL, ACCRA

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Detection and genotyping of *Toxoplasma gondii* in HIV infected individuals and blood donors at the Korle-Bu Teaching Hospital Accra Ghana. *T. gondii* is an obligate intracellular protozoan parasite which causes the disease toxoplasmosis. *T. gondii* infects through the gut, lung or broken skin. Cats (primary host) excrete oocysts, but the ingestion of poorly cooked infected meat by humans may also be as important as contact with cat feaces. In humans, the oocyst release trophozoites, which migrate widely with the prediction for eye, brain and muscle. Infection is live long and HIV may reactivate it. Purpose of study Detect and genotype *T. gondii* found in HIV infected individuals and blood donors visiting the Korle Bu Teaching Hospital Accra Methodology Blood samples (2-3ml) were collected from volunteers of HIV individuals and blood donors. DNA was extracted from the blood samples using the Qiagen blood and tissue kit. Nested (PCR) was performed using Sag 3 and Gra 6 primers. Samples that tested positive for Toxoplasmosis was further taken through restriction fragment length polymorphism using *Nci* I and *MSel* I restriction enzymes. To find the genotype that was present. Major findings Out of the 150 screened for HIV infected individuals 67/150 were positive for *Toxoplasma* representing 44.7%. Using Ncil and Msel restriction enzymes for genotyping 3/150, 2% positive for type I and 60/150, 40% for type II. The blood donors had 5/152, 3.3% positive for *T. gondii* and all were type II. In conclusion, the type II was common among the two groups, a few of types I was also found. The HIV infected individual who were found positive had their $CD4^+$ T cell count 0>200. A number of supposedly healthy blood donors were infected with *T. gondii,* but the blood donors donating at the blood bank are not been screened for *Toxoplasma gondii* and therefore there is a need to screen the blood donors before blood is been donated especially to the immunocompromised individuals.

887

PREVALENCE OF *TRICHOMONAS VAGINALIS* AMONG PREGNANT WOMEN IN KINSHASA, DEMOCRATIC REPUBLIC OF THE CONGO

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Trichomonas vaginalis is a flagellated protozoan causing sexually transmitted infection extremely common among the poor. It is cosmopolitan and is spread when hygiene measures are defective. It is also a cofactor in the transmission of HIV, a major cause of vaginitis in pregnant women and responsible for premature delivery, premature rupture of membranes, low birth weight children and infection of newborn. So we wanted to determine the prevalence of *T. vaginalis* among pregnant women. We conducted a cross-sectional study from November to December 2012 among pregnant women in Kinshasa. A sample of vaginal secretions swab was done, fixed on a slide and stained with Giemsa. Of 412 women enrolled, 96 patients were infected with *T. vaginalis* or 23.3%, whose average age was 28 ± 6 years. The infection was common among married women (55.6%), with a status of monogamous marriage (59.5%) whose spouse was trader (34.5%).

888

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN WILD BOARS IN SOUTH KOREA

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Toxoplasma gondii is an obligate intracellular protozoan parasite which is a significant human and veterinary pathogen. The wild boar (*Sus scrofa coreanus*) is regarded as a good indicator for monitoring environmental contamination with *T. gondii*. The purpose of this study was to survey the prevalence of antibodies against *T. gondii* in wild boars from South Korea. Blood samples were collected from 426 wild boars, which had been captured during 2008-2012 hunting seasons. Antibodies to *T. gondii* were detected in 152 samples of the 426 animals, indicating an overall seroprevalence of 35.7%. No correlation was found for any pairs between the prevalence of toxoplasmosis and the density of wild boar, or the rate of forest area (p>0.05 for all pairs).

889

ISOSPORA BELLI INFECTION WITH CHRONIC DIARRHEA IN AN ALCOHOLIC PATIENT

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Chronic diarrhea with a 35 kg weight loss (75 kg to 40 kg) occurred during 2 years in an alcoholic patient was diagnosed with *Isospora belli* infection in the Republic of Korea. The patient, a 70-year old Korean male, had been a heavy drinker for more than 30 years. He was admitted to the Seoul National University Hospital because of long-standing diarrhea and severe weight loss. He had an increased white blood cell (WBC) count with high peripheral blood eosinophilia (36.8-39.9%) and lowered protein and albumin levels but without any evidence of immunosuppression. A parasitic infection was suspected and fecal examination was repeated 3 times with negative results. Peroral endoscopy with mural biopsy was performed in the upper jejunum. The biopsy specimens revealed villous atrophy with loss of villi together with various life cycle stages of *I. belli,* including trophozoites, schizonts, merozoites, macrogamonts, and microgamonts. The patient was treated successfully with oral doses of trimethoprim 160-320 mg and sulfamethoxazole 800-1,600 mg daily for 4 weeks. A follow-up evaluation at 2.5 years later revealed marked improvement of body weight (68 kg), increased protein and albumin levels, and normal WBC count with low eosinophils (3.1%). This is the first clinical case of isoporiasis with demonstration of various parasitic stages in the Republic of Korea.

890

ANTIPROTOZOAL ACTIVITY OF DEFENSINS

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Invertebrate defensins are part of the cysteine-stabilized alpha-beta superfamily that also includes defensins from plants and fungi, and some arthropod toxins. Defensins are usually considered to be most effective against bacteria, but some also show antifungal and/or anti-*Plasmodium* activity. Several arthropod species (*Aeshna cyanea, Drosophila melanogaster, Pandinus imperator*, and *Phrormia terranovae*), express defensins/defensin-like peptides with activity against malaria parasites. In addition, the tick *Haemaphysalis longicornis* expresses a defensin called longicin that inhibits transmission of *Babesia.* Both *Plasmodium* and *Babesia* are vector-borne blood parasites, suggesting these defensins might have evolved in the context of vector-pathogen interactions. However, a defensin-like peptide from *Anaeromyxobacter dehalogenans* also shows anti-*Plasmodium* activity, leading us to hypothesize that these peptides may have a more general antiprotozoal activity. A short sequence motif (five amino acids) in the m-loop has been hypothesized to be the basis of this activity. If this motif does confer antiprotozoal activity, we would expect to see it in defensins from a broad range of taxa and not limited to those with vector potential. Using information available from online, public sequence databases, we identified putative defensins with the hypothesized antiprotozoal motif in non-arthropod taxa, including nematode and tardigrade species. Studies with recombinant peptides are in progress to determine the spectrum of defensin antiprotozoal activity and verify that the hypothesized sequence motif is the biological basis for that activity.

891

ASSOCIATIONS OF ENTERIC PARASITE INFECTIONS WITH ROTAVIRUS-NEGATIVE DIARRHEA IN CHILDREN UNDER FIVE YEARS: A CASE CONTROL STUDY IN RURAL DISTRICT IN ECUADOR

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There are few data on the associations between enteric parasite infections and diarrhoea. To explore the associations between common enteric parasites and diarrhoea in pre-school children, we did a case control study of children aged 5 years or younger in a rural District of Ecuador. Children aged 6 months to 5 years were recruited at a pediatric outpatient clinic in the town of Quininde, Esmeraldas Province. Cases were children presenting with acute diarrhea (3 or more liquid stools in the previous 24 hours) that was negative for rotavirus infection by enzyme immunoassay (Prospect Rotavirus, Oxoid, UK), an assay that has bene calibrated to detect viral loads indicative of roatvirus diarrhea, and controls were healthy asymptomatic children being reviewed at the same clinic. A stool sample was collected from all children and was examined using the Kato-Katz and formol-ether concentration methods for *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm infections. An aliquot of stool was stored at -20C and analysed by real-time PCR for the presence of DNA for *Strongyloides stercoralis*, *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium*. We evaluated 168 diarrhoea cases and 171 asymptomatic controls. The prevalence of infections among cases and controls were: *A. lumbricoides* (cases 11.1% vs. controls 9.3%); *T. trichiura* (9.3% vs. 9.3%); *S. stercoralis* (1.8% vs. 1.2%); *G. lamblia* (34.5% vs 28.1%), *Cryptosporidium* (4.08 vs. 0.6%, P=0.017). Only 1 child was infected with hookworm and none with *E. histolytica.* Although *Cryptosporidium* infection was detected in a small proportion of children aged under 5 years with mild to moderate diarrhoea, it was the only enteric parasite infection measured that was associated with diarrheal illness in our study population.

892

DETECTION OF ACUTE TOXOPLASMOSIS IN PREGNANT WOMEN BY INDIRECT HAEMAGGLUTINATION AND CONVENTIONAL NESTED PCR IN AL-MADINAH AL-MUNAWARAH, SAUDI ARABIA

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Difficulties met during interpretation of serological tests carried out in pregnant women to detect primary maternal infection with toxoplasmosis during pregnancy implies more and more frequent use of the molecular technique, Polymerase Chain Reaction (PCR). To evaluate the degree of correlation between the results of a routine serological test and the *Toxoplasma gondii* genome in mother blood, conventional nested PCR a rapid, sensitive and specific molecular diagnostic technique was applied on 100 pregnant women from Al-Madinah Al-Munawarah, KSA, whose blood samples were analyzed for the presence of B1 gene of *T. gondii*. The presence of IgG and IgM *T. gondii* antibodies were analyzed by indirect haemagglutination (IHA) assay. The study revealed no agreement between the results of IHA and PCR. *T. gondii* genetic material in blood was found in 41 (41%) samples. IgG was detected in 10 cases of this PCR-positive samples. IgM was also detected in 6 of 10 samples. On the other hand, 59 (59%) cases were PCR-negative ; 32 of them were serologically positive and the remaining 27 cases were serologically negative. In a conclusion, the study clarifies the need of a confirmatory assay in addition to serology for detection of primary acute toxoplasmosis (misdiagnosed cases) in pregnant women. Nested PCR (semi quantitative) amplification of the B1 gene of *T. gondii* using whole blood is a rapid, sensitive and strain specific molecular diagnostic technique and cosiderable a value tool for establishing the diagnosis of *T. gondii* infection in adult females before or during pregnancy in Al-madinah al-Munawarah, KSA. Our future study to apply the Real Time PCR (RT-PCR) technique for fast detection of acute toxoplasmosis cases in Al-Madinah AL-Munawarah, KSA.

893

DEVELOPMENT OF REAL-TIME PCR FOR THE QUANTITATIVE AND SPECIFIC DETECTION OF *BABESIA DUNCANI* IN BLOOD

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Babesia sp. are obligate protozoan parasites of red blood cells. Transmission to humans occurs through bites from infected ticks or blood transfusion. Infections with *B. microti*, which are endemic in the Northeast and upper Midwest regions of the United States, account for the majority of the reported cases of babesiosis in the US. The incidence of this disease is much lower in other US regions and is usually caused by the more recently described species *B. duncani*. The current gold standard for detection of *Babesia* is microscopic examination of blood smears. Recent PCR-based assays, including real-time PCR targeting the nuclear 18S ribosomal RNA gene, have been developed for *B. microti*. On the other hand, molecular assays that detect and distinguish the related *B. duncani* species are lacking. The greatest amount of sequence variation among related species of eukaryotes occurs within the internal transcribed spacer (ITS) regions of nuclear ribosomal RNA. Thus, in the present study, we targeted the ITS regions of *B. microti* and *B. duncani* to develop sensitive and species-specific real-time PCR assays. The assays were shown to discriminate *B. duncani* from *B. microti* and resulted in a limit of detection of 100 gene copies. Moreover, ITS real-time PCR for *B. duncani* was diagnostic in DNA extracted from blood of experimentally infected hamsters, detecting infections of low *parasitemia* that may potentially go undetected by microscopic examination (i.e., ≤0.01 % infected red blood cells). In summary, we have developed a sensitive and specific quantitative real-time PCR assay for the detection of *B. duncani* in blood. Our method could be used as a sensitive approach to monitor the progression of *parasitemia* in rodent models of infection as well as serve as a useful molecular test in blood screening.

894

DEVELOPMENT OF A QPCR-BASED METHOD TO QUANTIFY THE RATIO OF HOST-TO-PARASITE DNA IN *THEILERIA PARVA*-INFECTED HOST LYMPHOCYTES

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Theileria parva is a tick-transmitted intracellular apicomplexan pathogen of cattle in sub-Saharan Africa. It causes East Coast fever (ECF), an acute fatal disease that kills over one million cattle annually, imposing a tremendous burden on many smallholder African farmers for whom cattle is the primary source of food and income. Conventional and quantitative real-time PCR (qPCR) are commonly used for the detection of parasite piroplasms in tissue biopsies but quantification is generally not pursued. One of the primary challenges to relative quantification is the miniscule amount of *T. parva* DNA in mixed DNA samples, in which host DNA prevails by >5 orders of magnitude. However, the ratio of host-to-parasite DNA in a sample is essential to determine the feasibility of obtaining high quality parasite genome sequence data through whole genome sequencing of samples of *T. parva*-infected host cells. We have developed an absolute quantification method based on qPCR where recombinant plasmid vectors containing genes specific for *T. parva* and *Bos taurus* are used as the quantification standards. The highly accurate detection of the number of target gene copies within the sample was achieved using two different primer sets, which were specifically designed to amplify the *T. parva* homolog of the *Plasmodium falciparum* apical membrane antigen 1 (AMA1) gene, as well as the bovine gene hypoxanthine phosphoribosyltransferase 1 (HPRT1). The qPCR values were then used to determine genome copy number and the genomic DNA equivalence ratio, given the relative size of the host and parasite genomes. Quantification of parasites combined with genomic equivalence ratio determination provides a simple and reliable method of assessing *T. parva* loads, which is essential for next-generation sequencing applications and may also play a vital role in studying host-parasite relationship and treatment efficiency.

895

SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTH INFECTIONS AMONG PRE-SCHOOL AGED CHILDREN IN MBITA, WESTERN KENYA

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Recent surveys have shown large numbers of preschool-aged children (PSAC) (≤5 years) infected with *Schistosoma mansoni*, yet PSAC are typically excluded from mass treatment campaigns. Our study determined the prevalence and intensity of *S. mansoni* and soil-transmitted helminth (STH) infections and association between infection and growth outcomes in children aged 1-5 years in Mbita, western Kenya. A total of 1,458 PSAC (712 boys, 746 girls) were examined. Single stool samples were examined by Kato-Katz for *S. mansoni* and STH eggs. Hematuria was used as a proxy indicator of *S. haematobium* infection and hemoglobin concentration was determined to assess anemia (< 10g/dL). Growth morbidities among

PSAC were determined from the WHO Child Growth Standards. Overall prevalence of *S. mansoni*, *S. haematobium* and STH was 28.2%, 31.4% and 3.9%, respectively. Of the 396 stool samples positive for *S. mansoni*, egg density was 258.9 ± 569.1; 241 (60.8%), 98 (24.8%) and 57 (14.4%) were light, moderate and heavy infections, respectively. The youngest infected child was one year of age. Stunted growth was observed in 21% of PSAC, of whom 7.4% were severely stunted. Underweight and wasting were observed in 8.8% and 3.1% of PSAC, respectively, and 22.7% of PSAC were malnourished. The prevalence of anemia, independent of infection (n = 1,165), was 30.2%; 67.1%, 29.6% and 3.4% had mild, moderate and severe anemia, respectively. Growth morbidities and anemia were not associated with *S. mansoni* infection. Our findings add to evidence that young children are at risk for *S. mansoni* infection, and that infections debut before school enrollment age in high endemic areas. With acquisition of infection early in life, children might not receive first treatment for up to 4 years after infection if present deworming policies are not revised. PSAC are a potential reservoir for transmission, emphasizing the need for their inclusion when designing control strategies.

896

PUROMYCIN ACTIVITY AGAINST *SCHISTOSOMA MANSONI* FOR DEVELOPMENT OF AN ANTIBIOTIC SELECTION SYSTEM MEDIATED BY RETROVIRAL TRANSGENESIS

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Drug selection of transgenic schistosomes would be desirable as it would provide a means to enrich for populations of transgenic worms. We recently demonstrated that Murine Leukemia Virus (MLV) transduced schistosomes expressing neomycin phosphotransferase (NeoR) could be rescued using the aminoglycoside antibiotic, G418. In addition, after infecting snails with miracidia hatched from MLV-transduced eggs, sequencing of genomic DNA from cercariae released from the snails revealed the presence of transgenes, demonstrating that transgenes had been transmitted through the asexual developmental cycle, and confirming germline transgenesis. Moreover, the germline-transmitted transgenes encoding NeoR rescued cultured schistosomules from toxicity of the antibiotic G418. However, the aminonucleoside antibiotic puromycin has been shown to be faster and more efficient than G418 in selecting transgenic vertebrate cells (i.e. within 48 h). Accordingly, here we tested schistosome sensitivity to puromycin for eventual use in deriving selection of transgenic schistosomes via transduction of eggs with MLV carrying the puromycin resistance marker (PuroR). Schistosomules, eggs from liver or laid *in vitro* by adults, and sporocysts of *Schistosoma mansoni* were cultured in increasing concentrations of puromycin. Media and antibiotic were periodically replaced, and schistosomules and sporocysts were scored as live or dead by dual-fluorescence bioassay. Viability of schistosome eggs isolated from liver was evaluated by an egg hatch assay on days 5 and 10, whereas the development of the eggs laid *in vitro* was monitored microscopically every day and by egg hatch assay on day 7. Although eggs were insusceptible to G418, the developmental stages examined here were sensitive to puromycin. These findings will facilitate not only 'dual selection' of schistosomes with G418 and puromycin, but also the enrichment of MLV-transduced eggs and sporocysts that can be reintroduced in the life to cycle of the parasite augmenting the efficiency of the transgenic approach.

897

AN UNCEASING PROBLEM: PREVALENCE AND RISK FACTORS OF SCHISTOSOMIASIS AMONG CHILDREN IN YEMEN

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Schistosomiasis, one of the most prevalent neglected tropical diseases, is a life-threatening public health problem worldwide. In Yemen, schistosomiasis is the second leading cause of death, after malaria, with an estimated 3 million people are infected. This study aims to determine the current prevalence and associated risk factors of schistosomiasis among children in rural Yemen. Urine and fecal samples were collected from 400 children. Urine samples were examined using filtration technique for the presence of *Schistosoma haematobium* eggs while fecal samples were examined using formalin-ether concentration and Kato Katz techniques for the presence of *S. mansoni* eggs. Demographic, socioeconomic, behavioral and environmental information were collected using a pre-tested questionnaire. Overall, 31.8% of the participants were found to be positive for schistosomiasis; 23.8% were infected with *S. haematobium* and 9.3% were infected with *S. mansoni*. The prevalence of schistosomiasis was significantly higher among children aged > 10 years compared to those aged ≤ 10 years ($P < 0.05$). Multivariate analysis confirmed that presence of other infected family member, low household monthly income, using unsafe sources for drinking water, living nearby stream/spring and living nearby pool/pond were the key factors significantly associated with schistosomiasis among these children. In conclusion, these findings support an urgent need to start an integrated, targeted and effective schistosomiasis control programme with a mission to move towards the elimination phase. Besides periodic drug distribution, health education and community mobilisation, provision of clean and safe drinking water, introduction of proper sanitation are imperative among these communities in order to curtail the transmission and morbidity caused by schistosomiasis.

898

CLINICAL AND ULTRASONOGRAPHIC CORRELATES OF HEPATOSPLENIC SCHISTSOMIASIS AMONG CHILDREN AND ADULTS IN A *SCHISTOSOMA MANSONI* HYPERENDEMIC RURAL AREA OF ZAMBIA

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The aim of schistosomiasis control programs is to reduce disease morbidity. However, comprehensive evaluation of these programs using available morbidity assessment tools is limited. Here we present clinical and ultrasonography correlates of hepatosplenic schistosomiasis among children and adults in a rural area of Zambia illustrating the limitations in these tools. Seven hundred fifty-four community members (159 children and 595 adults) from four rural locations (Mwadasengo, Luampa, Namando and Mangango) of Kaoma District had clinical asssesmments (September-October 2012) and were tested for *Schistosoma mansoni,* other helminthes, malaria, and haemoglobin status. Ultrasonography was done in 710(94%) participants to check for liver abnormalities. Of the 717 screened for parasitic infections, *Schistosoma mansoni*, hookworm, and malaria infection prevalence were 42%, 26.9%, and 6.5%, respectively. Twelve percent had hookworm-*S. mansoni* co-infection, 5.2% *S. mansoni*-malaria and 1.95% *S. mansoni*-hookworm-malaria multiple infections. On ultrasonography 72.4% had no periportal fibrosis (PPF),14.4% had mild while 7.2% and 6% had moderate and severe PPF, respectively. On logistic regression, sex-female (odds ratio [OR] = 2.0; 95% CI= 1.26,

3.34), age ≥44 (OR = 5.86; 95% CI = 1.70, 20.25), area-Namando (OR = 4.73; 95% CI = 1.62, 13.86), and PPF-(p <0.001) were associated risk factors for splenomegaly while, intensity of infection and other parasitic infestations were not. On the other hand, only age group 34-44 (OR = 3.25; 95% CI = 1.50, 7.05) and age group ≥44 (OR = 3.20; 95% CI =1.43, 7.14) were strong associated risk factors for PPF. Of note was the strong correlation between PPF severity with ultrasonographic portal vein diameter (r=0.56, p-value <0.001) and weakly so for hepatomegaly(r=0.29 p <0.001).Collectively, these findings affirm the limitations of the available schistosomiasis morbidity assessment tools. There is urgent need to devise standardized clinical criteria or indeed search for molecular markers for the accurate assessment of schistosomiasis morbidity.

899

DETERMINATION OF TEMPERATURE EFFECTS ON SUSCEPTIBILITY OF *BULINUS TRUNCATUS TRUNCATUS* SNAILS TO *SCHISTOSOMA HAEMATOBIUM* AND ASSOCIATED MORTALITY AND FECUNDITY

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One obstacle in laboratory maintenance of *Schistosoma haematobium* is inherent resistance among the intermediate host *Bulinus spp.*, where infection rates in the laboratory are as low as 30%. A previous study in the *B. glabrata/S. mansoni* system showed heat exposure of snails prior to parasite infection can alter susceptibility. We studied effects of increased temperature on the susceptibility of *B. t. truncatus* to *S. haematobium* by exposing them to heat (32ºC) prior to infection. Three distinct snail size groups were used: neonates (≤1-2mm length), pre-adults (≥2-3mm), and adults (>8mm). Over 10 weeks post-infection, we monitored infection rates and effects on fecundity of *B. t. truncatus*. Across size groups, an inverse relationship between size and susceptibility was observed. Comparison of ambient temperature vs. heat-treated snails in each size group found heat-exposure increased infection rates ~2-fold in neonate and pre-adult snails. Adult snails were relatively non-susceptible. In the neonate and pre-adult groups, increased susceptibility due to heat-exposure correlated with lower mortality compared to non-heat treated snails. During patency, the mean weekly fecundity ratio (#egg sacs/surviving patent snails) of heat-treated neonates and ambient temperature pre-adults was significantly less than matched uninfected snails (p<0.05). No significant difference was observed in the mean weekly fecundity ratio between patent ambient vs. heat-treated neonates and pre-adults. Our results suggest that temperature manipulation can alter susceptibility of young *B. t. truncatus* snails to *S. haematobium* and it may have a protective effect for survival. Heat-exposure affected neonates the most for increased susceptibility but also decreased fecundity in these snails. Although pre-adult snails exhibited less susceptibility than neonates, they tolerated heat stress better in terms of fecundity and mortality. These results have implications for optimization and improving propagation of the *S. haematobium* life cycle, which will be valuable for future schistosomiasis research.

900

HEPATOSPLENIC *SCHISTOSOMIASIS MANSONI* DISEASE BURDEN IN ZAMBIA: CLINICAL AND LABORATORY EVALUATION TO DETERMINE PHENOTYPES FOR CYTOKINE GENE-POLYMORPHISM ANALYSIS

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Globally, schistosomiasis is a chronic parasitic disease of major public health importance being endemic in 78 countries with at least 243 million people reported to have required treatment in 2011.Chronic *Schistosoma mansoni* infection can present into a wide spectrum of clinical syndromes with various degrees of severity. Several studies have delineated the aetiopathogenesis of chronic schistosomiasis disease syndromes to be due to schistosomal elicitation of cross-regulatory innate, Th1, Th2, and Th17 immune responses in the host. Immune responses attributable to *Schistosoma mansoni* infections are reported to be under host genetic control. Elucidating the immuno-genetic background to these immune responses, in the human host, will not only provide further understanding of schistosomiasis immunopathology but may herald novel effective ways of combating the disease. Here we describe the design, clinical and laboratory evaluations of a cross-sectional study cohort of individuals in a *schistosoma mansoni* hyperendemic rural Zambia set to segregate phenotypes for hepatosplenic schistosomiasis cytokine gene-polymorphism analysis.

901

EFFECT OF HISTONE DEACETYLASE INHIBITOR ON GENE EXPRESSION PROFILES AND HISTONE ACETYLATION IN *SCHISTOSOMA MANSONI*

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Histone modifying enzymes such as histone deacetylases (HDACs) have a key role in the epigenetic regulation of gene expression, and their potential as new therapeutic targets was evidenced with the apoptotic phenotype that resulted from *S. mansoni* HDAC inhibition by Trichostatin A (TSA). In order to characterize gene expression changes caused by *S. mansoni* treatment with TSA, microarray experiments were performed. Total RNA was extracted from schistosomula (LE strain) treated for 12, 24, 48 and 72 h either with 1µM TSA or with ethanol (vehicle) as a control. Triplicate samples were labeled with either Cy3 or Cy5 and hybridized to custom-designed Agilent oligoarrays. Gene expression analyses showed 870 (12h), 968 (24h), 703 (48h) and 188 (72h) genes with statistically significant differential expression (q-value<0.05) and fold-change >2. Ingenuity Pathway Analysis (IPA) showed gene networks significantly enriched with differentially expressed genes related to DNA replication, cell cycle, cell death and survival, protein synthesis and nucleic acid metabolism. At 12 and 24 h treatment, genes related to DNA replication, recombination and repair were up regulated. Interestingly, genes related to and regulated by Polycomb repressive complex suggest that this complex has a decreased activity after 12 h treatment of schistosomula, which could affect the chromatin methylation status. Moreover, western blotting with total protein lysates from adult worms treated with TSA showed hiperacetylation at H2A-Lys5, H2B-Lys12, H3-Lys9 and H4-Lys5. This study provides important data for understanding the response of the parasite to hiperacetylation of histones and regulation of gene transcription.

902

DIFFERENCES IN THE EXPRESSION OF HSP70 STRESS PROTEIN BETWEEN JUVENILE *BIOMPHALARIA GLABRATA* SNAILS THAT ARE EITHER SUSCEPTIBLE OR RESISTANT TO *SCHISTOSOMA MANSONI*

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The stress protein Heat shock protein 70 (Hsp 70) is constitutively expressed in the *Schistosoma mansoni* intermediate snail host, *Biomphalaria glabrata*. Susceptible stocks of *B. glabrata* allow miracidia to freely develop after penetrating the soft tissue parts of the snail host. Thus, after shedding its cilia plates the miracidium developme, United Statesnt in the susceptible NMRI snail, progresses from mother to daughter sporocyst stages, permitting asexual reproduction of the free- swimming cercarial stage. In the parasite- resistant juvenile snail, such as BS90, however, invading parasites are encapsulated and killed not long after penetration. Mechanism(s) shaping these outcomes involves the parasites ability to evade the snail's innate defenses. By Western blot analysis using anti-snail recombinant Hsp 70 antiserum, soluble protein extracts from NMRI and BS-90 snails exposed to *S. mansoni* for different time points was examined. Results showed early and strong induction of Hsp70 protein in susceptible but not resistant snails. These data will aid in understanding how a parasite -mediated stress response, involving transcriptional regulation of Hsp 70, and juvenile snail susceptibility helps with the development of intra-molluscan stages of *S. mansoni*.

903

THE FEASIBILITY STUDY OF THE *ONCOMELANIA* SNAIL DETERMINATION OF SCHISTOSOMIASIS BY GPS AND GOOGLE EARTH

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In endemic areas of schistosomiasis, the oncomelania snail survey is one the key measure to control schistosomiasis. The current *Oncomelania* snail survey lacks accuracy and repeatability of the snail spots, and manual workload is very heavy after the survey. To explore to reduce the manual workload and improve the accuracy by applying GPS and Google earth. This study used 3 methods to solve the problems: 1 Compare the feasibility on different positioning techniques such as GPS, maps, softwares in *Oncomelania* snail survey. 2 Synthesis of Positioning technology. 3 Feasibility study and case analysis: Systematic verification on Yangtze River bund. Finally, the study attained the following findings: 1 Use the Google earth to ascertain the environment in advance and decide the sampling techniques of snail survey. 2 Use the handheld GPS to locate the work track and the snail spot in particular and gather the surrounding photographs. 3 Import/export in batch the data of GPS(not input/export one by one), innovate the data expression and get the environment database and geographic information map. 4 The current location accuracy is about 50 meters, and this study limit the accuracy within 3 meters. The current data process usually needs 5 days, but this study just needs half a day. To conclude, this study makes the task of snail survey clearer, the location more accurate, the snail spot more repeatable, the data process more efficient, the snail distribution more direct, the data sharing more convenient, and lay a reliable basis for monitoring and early warning of snail status.

904

RISK ASSESSMENT OF ACUTE SCHISTOSOMIASIS OUTBREAK AROUND THE RENOVATION OF YANGTZE RIVER BUND BY DECISION TREE METHOD

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It is generally believed that it is not suitable for the breeding of snails after the hardening and renovation of Yangtze River bund. With effective control of schistosomiasis in the past years, the vigilance of residents living on the banks of the Yangtze River is decreasing year by year. But snail reproduction (no infectious) was founded, The objective of the study is to make a scientific evaluation design by the decision tree method, the observational method and the bowknot method. To achieve the above objectives, the authors analyze characteristics of mainstream people on the Yangtze River bund, determined the existing risk factors, classified relevant risk factors by observational, decision tree and bowknot methods respectively. Finally, the authors got the following risk assessment study design: Firstly, social behavioral observational method was used to investigate the spatial pattern of mainstream people on the Yangtze River bund. Secondly, systematic sampling and GPS was combined to accurately position infectious snail distribution on the Yangtze River bund. Thirdly, the Delphi method and risk matrix method was applied to assess the occurrence possibility and harmfulness of acute schistosomiasis infection. After conducting a trial by using the above methods on the Yangtze River bund,this scheme proved feasible, and the main target groups were identified,the snail distribution was clear,and the results risk evaluation is reliable.

905

CHARACTERIZATION OF THE BAS-CONGO VIRUS GLYCOPROTEIN AND ITS FUNCTION AND USE IN PSEUDOTYPED VIRUSES

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The novel rhabdovirus Bas-Congo virus was first identified from a survivor of a small hemorrhagic fever outbreak in the Bas-Congo province of the Democratic Republic of Congo (DRC). However, it was not possible to culture the virus and a reverse genetics assay is still under development. Using only the BASV glycoprotein (BASV-G) we established a pseudotype system based on a glycoprotein-deficient vesicular stomatitis virus (VSV) vector to study BASV-G driven membrane fusion and viral entry into target cells without replication-competent virus. In vitro studies revealed that BASV-G displayed a broad tissue and species tropism similar to its well-studied relative VSV-G. While BASV-G mediated membrane fusion was pH-dependent, acidification in the absence of a target membrane did not lead to inactivation of the viral fusion protein. This suggests that the conformational changes induced in BASV-G by low pH are fully reversible. This data and structural features identified by comparative sequence similarity analyses with other rhabdovirus glycoproteins support the classification of BASV-G as a class III viral fusion protein. Notably, transition of BASV-G into the fusion active conformation required a lower pH and higher temperatures than those observed for VSV-G. Another distinction from VSV-G was the finding that BASV-G contained high-mannose glycans that allowed binding to certain C-type lectins, thereby enhancing its attachment to target cells. The BASV-G pseudotype assay described in this study will be an important tool in the absence of an infectious cell culture assay for BASV. It will facilitate future investigations of BASV-G mediated cell entry and its inhibition, as well as serology testing required to uncover the prevalence and importance of BASV as a potential novel human pathogen in the DRC and throughout Central Africa.

906

BIOSAFETY AND BIOSECURITY CHALLENGES IN CONTROLLING VIRAL HEMORRHAGIC DISEASES OUTBREAKS IN UGANDA

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Uganda lies in a region where many viral diseases, leading to a lot of debilitation and deaths, have emerged in the recent past. Lying at the equator and being at the confluence of the Equatorial Forest zone of West Africa and the Tropical Savannah zone of Eastern Africa it hasa good climate, many freshwater rivers and lakes, and a diverse flora and fauna favourable for many insect vectors and reservoirs of viral infections. Particularly alarming are the VHFs: Ebola Virus fever, Marburg Virus fever, Yellow fever, Dengue fever and others that have been recorded in Uganda in the recent past. A lot of training in biosafety has been emphasized over the years but biosecurity is a new term and often confused with biosafety. Several programs to promote biosafety and biosecurity have been introduced in the country. However, there are many challenges to implementation of these programs which are developed in the western world. There are limited resources and infrastructure to support and implement them in a systematic and sustainable manner. Simply getting core concepts and procedures into the hands of those working with biological agents and processes is a significant challenge. Many of the facilities handling infectious agents in developing countries were built more than 30 years ago, with little or limited provision for biosafety and biosecurity in terms of both design and practice. The conditions found in the majority of these facilities remain shocking to those operating in laboratories in developed countries. Shortcomings in capacity, equipment and human resources pose a weak link in the chain of control against the misuse and abuse of infectious agents to inflict harm. Other challenges include: short comings in bioethics and accountability, poor remuneration of personnel, loss of research materials, loss of collaboration opportunities, loss of funding opportunities, loss of training opportunities, loss of property rights and loss of control of our activities and facilities. There is need to increase governments efforts to improve the quality of facilities. We have been training personnel nationally and internationally; we have improved our biosafety and biosecurity in institutions handling highly infectious pathogens. We are developing a national policy on biosafety and biosecurity and we have also formed a biosafety and biosecurity association to address some of the challenges.

907

FATAL NEUROLOGICAL DISEASE IN RATS AND NON-HUMAN PRIMATES AFTER INHALATIONAL EXPOSURE TO RIFT VALLEY FEVER VIRUS

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Rift Valley Fever virus (RVFV) outbreaks occur in Africa and the Middle East, resulting in severe disease in livestock and human populations. In humans, RVFV causes a self-limiting febrile illness, but some people develop severe complications including encephalitis. RVFV is infectious by many routes, including inhalation. Animal models that mimic human disease can be used as tools to understand the pathogenesis and test the efficacy of potential countermeasures. Up to this point, there have been no well-characterized rodent or NHP models for the encephalitic form of RVF. In the current studies, ACI inbred rats, African Green Monkeys (AGM), and marmosets were exposed to small particle aerosols containing RVFV, and all developed fatal neurological disease. ACI rats develop a fever and encephalitis after either s.c. inoculation or inhalation of RVFV, although 1,000 - 10,000-fold more virus is required to cause lethality by s.c. infection compared to aerosol. ACI rats have little peripheral virus replication but high levels of virus are found in the brain. Granulocytic leukocytosis was seen in the blood during the course of infection. Using radiotelemetry, both NHP species demonstrated a marked fever response after infection. Five out of six (5/6) AGM and four of eight (4/8) marmosets developed neurological signs (drooling, unsteady gait, seizures) and became moribund. The median lethal dose in marmosets was estimated to be 3,500 pfu. This is the first documented evidence of reproducible, severe disease seen in NHPs after infection with RVFV. High levels of infectious virus were found in brain and spinal cord, and both species displayed elevated white blood counts, which consisted primarily of granulocytes. Pathological findings confirmed viral encephalitis in both AGMs and marmosets. In conclusion, ACI rats, AGM, and marmosets all develop neurological disease after aerosol exposure to RVFV that is similar to that seen in humans. All can serve as appropriate tools to understand the pathogenesis and evaluate therapeutics against inhalational Rift Valley Fever.

908

SEROEPIDEMIOLOGICAL STUDIES AND ASSESSMENT OF PREVELANCE OF TICK-BORNE ENCEPHALITIS AND CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN KAZAKHSTAN

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In Kazakhstan, the Almaty province is endemic for tick-borne encephalitis (TBE), but has no human cases of Crimean-Congo hemorrhagic fever (CCHF) registered. The Kyzylorda province is endemic for CCHF, but has no human cases of TBE registered. The objectives of the study were to assess CCHF and TBE antiviral antibodies in individuals residing in endemic and non-endemic areas, and to assess CCHF and TBE infection rates among ticks collected from those same areas. We tested 500 serum samples from healthy individuals residing in four regions of Almaty province and five regions of Kyzylorda province for the presence of IgG antibodies using ELISA. We also examined 5463 ticks (264 pools) in the genera *Hyalomma*, *Haemaphysalis* and *Dermacentor*, collected in the same regions, for the presence of viral antigen using ELISA and viral RNA using RT-PCR. In the Almaty province, IgG antibodies to TBE virus were found in 18% of all serum samples. There were no samples positive for CCHF antiviral antibodies. CCHF-infected ticks were found in one region and TBE infected ticks were found in two regions. In the Kyzylorda province, IgG antibodies to TBE virus were found in 2.4% of all serum samples. 7.3% of all serum samples in this province were positive for CCHF, while 1.7% samples were positive for both TBE and CCHF. Prevalence of TBE virus infection among ticks collected in all regions of Kyzylorda province varied from 0% to 1.15%, while CCHF prevalence was between 0% and 2.16%. Thus, antibodies to TBE virus were found in individuals residing both in endemic Almaty province and non-endemic Kyzylorda province. CCHF antiviral antibodies were detected only among samples from Kyzylorda. The highest infection rate of TBE and CCHF in ticks and the highest level of presence of TBE and CCHF antibodies in humans coincided in the same regions. Clearly, the extent of human exposure to TBE virus has been underestimated in Kazakhstan.

909

DEVELOPMENT OF A MOSQUITO TRAP THAT USES SUGAR FEEDING TO DETECT EASTERN EQUINE ENCEPHALITIS VIRUS

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Eastern equine encephalomyelitis virus (EEEV) is the most pathogenic arbovirus endemic to the USA. Prevention of infection relies upon transmission surveillance and community-wide prevention measures to prevent the spread of the virus to humans. Many counties in Florida cannot afford the costs associated with thorough active surveillance, including testing of wild birds, sentinel chickens, and mosquito pools. For mosquito surveillance, sample size is extremely important due to low infection rates in mosquito populations. Current methods rely on mosquito pools with no greater than 50 mosquitoes and can be costly and time consuming. We designed a surveillance system that exploits virus secretion in saliva during sugar feeding by mosquitoes. Modified collection chambers of CO_2-baited traps are supplied with honey-coated nucleic acid preservation cards. Mosquitoes that feed upon honey expectorate viral particles onto the card which are then inactivated and preserved by the card. RNA extracted from the cards can then be screened via RT-PCR for arboviruses. This method will allow us to screen more mosquitoes at a time, decreasing the amount of labor and cost. In field trials, we found that 1) the modified traps captured as many females with a similar species distribution as did standard CO2-baited CDC light traps; 2) nearly all females (91.4%) in traps fed on honey; and 3) traps could run unattended for 3 consecutive days on a single battery and CO_2 tank. Experimental inoculations of EEEV onto honey coated preservation cards demonstrated that viral levels down to 1 PFU were detectable for up to seven days. Additional field trials are currently in progress and will be included in the data presented at the conference.

910

A FILOVIRUS MARATHON: EPIDEMIOLOGICAL AND LABORATORY RESPONSES TO TWO OUTBREAKS OF MARBURG AND EBOLA HEMORRHAGIC FEVER, UGANDA, OCTOBER-NOVEMBER 2012

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On October 18, 2012, blood specimens from three patients in the Kabale District of Uganda were laboratory-confirmed for Marburg virus infection. National and international agencies responded by rapidly establishing active case finding, contact tracing and follow-up, a field diagnostic laboratory, an isolation ward, and outreach to the affected communities. Marburg Hemorrhagic Fever (MHF) case-patients were defined as confirmed cases with laboratory detection of Marburg virus or antibodies, while probable cases had clinical illness and epidemiologic linkages in the absence of laboratory testing. A total of 26 confirmed and probable MHF cases in 3 districts were identified, with the first illness onset dating to July 2012--3 months prior to the outbreak's detection. Case fatality rate was 58%. There were two distinct chains of transmission, both occurring in Ibanda district at the same time. Sequence analysis of patient specimens from both chains found 99.9% matching in sequence identity of virus strains, indicating a single outbreak. In the midst of the MHF response, 2 patients with Sudan virus infection were detected in the Luwero district of Uganda on November 13. Response efforts were quickly diverted to this outbreak. A total of 7 Ebola Hemorrhagic Fever (EHF) case-patients were identified, 6 of which were laboratory-confirmed, and 4 who died (Case fatality rate = 57%). In contrast to the MHF outbreak, the first identified EHF case-patient had illness onset approximately 1 month prior to the outbreak's detection. In both outbreaks, timely mobilization of resources occurred, and all case-patients were put into isolation within 2 weeks. The origin of the epidemic--presumed to be zoonotic transmission of filovirus from infected animals to humans--was investigated but not identified in either outbreak. Early identification and halting of direct human-to-human transmission contributed to the successful containment of these filovirus epidemics; the presence of diagnostic capacity in country, coupled with clinician awareness was crucial to prevention of additional cases and deaths.

911

BURDEN AND SEVERITY OF INFLUENZA INFECTIONS IN YOUNG ANDEAN CHILDREN

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We conducted a prospective household-based study of acute respiratory illness (ARI) among children in the rural Peruvian Andes. We used these data to characterize the burden of influenza-associated ARI (IA-ARI), identify risk factors for influenza infections, and compare the clinical characteristics of IA-ARI with other ARI etiologies. We followed a dynamic cohort of 892 children age <3 years in the highland villages of San Marcos Province, Cajamarca, Peru, from May, 2009 to September, 2011. Trained field workers visited the home of each child weekly, collected data on respiratory symptoms, and assessed symptomatic children for danger signs including: wheezing, retractions, grunting, nasal flaring, stridor and tachypnea. Nasal swabs collected from children with ARI (cough or fever) were analyzed for respiratory viruses by monoplex real-time RT-PCR. The incidence of IA-ARI by influenza types (A, B and C) was calculated by Poisson estimation. Characteristics of IA-ARI and non-IA-ARI were compared using two-proportion z or Wilcoxon rank-sum tests. Influenza vaccination status for each child was determined. The overall incidence of IA-ARI was 34/100 child-years. Influenza A incidence was highest (20/100 child-years), followed by influenza B (9/100) then influenza C (5/100). Influenza circulated seasonally, with moderate peaks in July - October, 2009 and June - December, 2010, and a small peak in August-September, 2011. Pandemic H1N1 accounted for 46% of influenza A: 89% (33/37) in 2009, 27% (30/111) in 2010, and 100% (7/7) in 2011. When compared with non-IA-ARI, IA-ARI were longer in duration (median 6.5 vs. 5 days, $p < 0.001$), with more days of fever (median 2 vs. 1, $p<0.001$), cough (5 vs. 4 days, $p<0.001$), and malaise (median 2 vs. 0 days, $p < 0.001$). IA-ARI were not more frequently associated with danger signs (6.9% of IA-ARI vs. 7.5% of others, $p=0.77$) or hospitalization (2.5% vs. 2.7%, $p=0.91$), but IA-ARI more frequently resulted in health care center visits (38% vs. 24%, $p < 0.001$). Influenza vaccination coverage for the observed seasons was too low to assess vaccine effectiveness. Influenza burden is high among Andean children and associated with longer illness duration and higher health care utilization than non-influenza ARI. Influenza C circulated in the study settings but its burden was low relative to other influenza types. Improvements in influenza vaccine delivery in this population are needed.

912

SYMPTOMATIC AND ASYMPTOMATIC NOROVIRUS INFECTIONS DURING THE FIRST THREE YEARS OF LIFE IN A TROPICAL ECUADORIAN BIRTH COHORT

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Noroviruses are recognized as a major cause of acute gastroenteritis worldwide. In some developed countries using rotavirus vaccination, noroviruses are the most common cause of pediatric hospitalization for gastroenteritis. With upcoming norovirus vaccines, it is important to understand the incidence of endemic, community-based disease and the development of natural immunity to norovirus infection and diarrhea. We monitored 194 Ecuadorian infants born from March-December 2009 from birth until 3 years by weekly phone calls, clinic surveillance, and stool collections during diarrhea episodes and diarrhea-free periods at scheduled home visits. Noroviruses were quantified by genogroup-specific real-time, quantitative PCR. 1360 samples were collected from 194 children over 3 years of life, with a mean of 8.68 samples per child. 432 (32%) samples were collected during episodes of acute diarrhea. Norovirus was detected in 248 (18%) of all samples, but was not found more frequently in diarrhea samples (79/432; 18.2%) than routine samples (169/ 928; 18.3%, p=0.973). However, viral loads (indicated by cycle threshold (Ct) values) were higher in diarrhea samples (median Ct = 27.9) compared to routine samples (Ct = 32.2; Wilcoxon rank-sum p = 0.004). The incidence of all cause diarrhea was 94.7/100 child-years (95% CI: 85.2 - 105.1) while that of norovirus-positive diarrhea was 21.5/100 child-years (95% CI: 17.2 - 26.8). 39% of norovirus strains were genogroup 1 and 61% were genogroup 2 (n = 248). Norovirus infection prevalence was strongly associated with age (p<0.001): 2% (4/176) at 0-5 months; 24% (185/764) at 6- 23 months; and 14% (59/420) 24-35 months. 3% (4/128) of children experienced a primary infection by 6 months, 35% (49/139) by 1 year, 62% (87/140) by 2 years, and 74% (104/140) by 3 years. Norovirus infections were strongly associated with age, and a majority of children had at least one infection by age 3. Although norovirus prevalence was not associated with disease, viral load was, with higher levels in diarrhea cases in this tropical community-based birth cohort.

913

TRANSCRIPTOME SEQUENCING REVEALS ELEVATED IMMUNE GENE EXPRESSION IN DEER MICE INFECTED WITH ANDES VIRUS

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Hantavirus cardiopulmonary syndrome (HCPS) is a cytokine-mediated disease with a high case-fatality rate that is caused by several New World hantaviruses. Each virus is naturally hosted by a principal rodent species without conspicuous disease, and once infected the rodents remain infected, perhaps for life. Deer mice (*Peromyscus maniculatus*) are the natural reservoir hosts of Sin Nombre virus (SNV), an etiologic agent of HCPS. Despite a helper T cell response that leads to high titered neutralizing antibodies, deer mice remain persistently infected. Deer mice are also susceptible to Andes hantavirus, which causes HCPS in South America; however, deer mice clear ANDV. We infected 5 deer mice with SNV and 5 deer mice with ANDV and examined lymph node cell antigen recall responses by deep transcriptome sequencing using the Illumina MiSeq and HiSeq platforms. We obtained about 1 million reads per sample from the MiSeq, and obtained a mean of 44 million reads per sample from the HiSeq with a range of 33 million to 54 million reads. We assembled the transcriptomes using Trinity. Analysis of MiSeq data from the two groups identified 80 genes that were significantly different between cultures from SNV and ANDV infections. All but two of the transcripts were more abundant in cultures from ANDV-infected deer mice than those from SNV-infected deer mice. Most of the transcripts have immune functions, while some are not known to have immune function. About half of the transcripts were related to interferon and/or antiviral responses, indicating that such responses occur with greater magnitude in deer mice infected with ANDV than SNV. We are continuing to analyze the HiSeq data to identify additional differentially expressed transcripts, identify differences between individual deer mice and to map the genes to functional pathways. Together with our previous work, these data suggest the magnitude of the immune response is substantially greater during ANDV infection compared to SNV infection, and may account for clearance of ANDV.

914

THE CONTRIBUTION OF HERD IMMUNITY TO THE EPIDEMIC CYCLES OF RIFT VALLEY FEVER VIRUS IN SOUTH AFRICA

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Rift Valley fever (RVF) causes devastating outbreaks in both humans and domestic animals, leading to significant morbidity, mortality and economic instability. While epidemics of RVF virus (RVFV) and correlate closely with climate and NDVI (normalized difference vegetation index) in East Africa, the climate models are less effective predicting outbreaks in southern or western Africa. We hypothesize that the herd immunity of ruminants (both wild and domestic) can affect the severity, duration and location of RVFV outbreaks. We developed a dynamic mosquito-host-infection model for integration within the current climate models to better predict the occurrence and spread of RVF epidemics in South Africa. Our model includes *Aedes* sp mosquitoes, which transmit RVFV transovarially, *Culex* sp mosquitoes, which spread RVFV from infected ruminants to susceptible ruminants or humans, and ruminants, including parameters such as age, previous exposure and vaccination. When forced with a combination of ENSO-like periodicity (4-7 year) for mosquito abundance and annual seasonal variation of the mosquito population, these equations can produce periodic epizootics of RVF in the ruminant population. This demonstrates that herd immunity may have an important role in the occurrence of an RVF outbreak, which likely changes depending on management regime (culling and vaccination) and the most recent RVF outbreak. This model, in conjunction with the climate models, could improve the prediction of outbreaks in regions with varying climate, unlike that of East Africa. This model could also potentially be used to predict the movements and likelihood of outbreaks under climate change scenarios.

915

RISK FACTOR ANALYSIS FOR NIPAH INFECTION IN BANGLADESH, 2004 TO 2012

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Since 2001, human Nipah encephalitis outbreaks have been identified almost yearly in Bangladesh. Over 70% of infected persons die. Raw or fermented date palm sap consumption and person-to-person transmission have been identified as major transmission pathways. However, many identified case-patients have had neither of these exposures based on epidemiologic surveys, suggesting alternative pathways of transmission that have not been identified in individual outbreak investigations due to limited statistical power related to small sample sizes. We conducted a risk factor analysis using data from all investigated 157 cases from 2004-2012 and their geographically-matched controls to maximize our power to identify rarer exposures associated with being a Nipah case. Using conditional fixed effects logistic regression, we first generated univariate models, adjusting for age, sex, date palm sap consumption and contact with a sick case. We then built multivariate models based on these results and selected the best fitting model using the Akaike information criterion; associations were considered significant at $p<0.05$. Median age of case-patients was 26 (range 3 months to 95 years) years, 48% were female, and 22% reported no exposure to date palm sap or contact with a Nipah case-patient. In the multivariate model, case-patients were 3.3 (95% CI:1.2 - 8.8) times more likely to live near trees where Pteropus bats visit at night and 3.5 (95% CI: 1.5 - 8.22) times more likely to travel outside their sub-district than controls; we found no significant associations with eating dropped fruits or contact with animals. Most Nipah case-patients die; finding knowledgeable proxy respondents to ascertain exposures of Nipah case-patients who travelled is difficult. The most likely explanation for the association with travelling is that exposures to date palm sap or other Nipah cases likely occurred during travel but went unreported. The association with bats around the house at night is likely another indicator of bat contamination of date palm sap. While pathways of transmission other than date palm sap consumption and person-to-person transmission may exist, our evidence suggests that these are not major contributors to human Nipah infection in Bangladesh. These findings underscore the importance of focusing Nipah prevention efforts on interventions to interrupt transmission through date palm sap consumption and person-to-person contact.

916

ANTIBODIES TO POLIO IN GAMBIAN SUBJECTS: DO OUR VACCINATION PROGRAMS PROTECT ENOUGH?

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Vaccination remains one of the most cost effective interventions in preventing serious infectious diseases, including polio. As we move toward polio eradication additional oral polio vaccine (OPV) doses have been included in the form of boosters and supplemental immunization campaigns, primarily given to children under the age of 5. It is important to assess the impact of such vaccine programmes not only in young children but also in older children and adults in order to minimise susceptibility. The concentration of polio antibodies in children above 5 years who received several OPV doses and that of young adults vaccinated several years earlier with fewer OPV doses has not been previously assessed in our setting. Such information is crucial to assess the impact any polio outbreak would have in The Gambia and in evaluating the need for alternative vaccination schedules to facilitate polio eradication. This study was undertaken determine antibody persistence in 5 to 6 year olds compared with adults in The Gambia. Samples from 223 healthy 5 to 6 year olds and 83 individuals 18years or above were tested for polio antibodies using an established seroneutralization assay against polio 1 and 3. Titres ≥ 1:8 were considered protective. Preliminary results show 95% and 85% of fully vaccinated 5 to 6 year old had protective antibody concentrations to Polio serotypes 1 and 3 respectively. Fewer adults had protective antibody concentrations to Polio 1 (85%) with less protection against Polio 3 (80%). In conclusion, data suggest that multiple OPV doses given to young children are producing the desired effect with some herd effect in adults. Some adults may however remain vulnerable.

917

EVIDENCE OF CIRCULATION OF SELECTED ARBOVIRUSES IN IJARA AND MARIGAT DISTRICTS, KENYA

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Arboviruses are transmitted by arthropods with humans becoming infected during blood feeding by infected mosquitoes, ticks and sandflies. Characterization of arbovirus circulation and transmission in industrialized countries has been well documented, but there are many knowledge gaps in developing nations. Entomological surveys conducted so far have indicated circulation of arboviruses of significant public health importance in *Aedes*, *Anopheles* and *Culex* species in vast populations in Kenya, suggesting the presence of competent vector systems. The human involvement in the transmission cycle of these viruses has however not been demonstrated. This study sought to determine the sero-prevalence of a range of arboviruses including; Chikungunya, Dengue, Sindbis, Sandfly Naples, Sandfly Sicilian, Uganda S, West Nile and Zika viruses in Ijara and Marigat Districts where vector surveillance has been done. A total of 351 patient serum samples were analyzed using IgG ELISA, of these 193 (54.9%) were male and 158 (45.1%) were female with age ranging between 3 and 73. The overall arbovirus prevalence was 53/351 (15.1%) with a prevalence of 7% (10/143) in Marigat and 21% (43/208) in Ijara. Of the positives, Flaviviruses were 69%, alpha viruses 29.6% and Bunyaviridae 1.4%. Uganda S virus was the most prevalent with 10%, followed by West Nile virus 6%, Sindbis 5%, Dengue 2%, Chikungunya 1.1%, Sandfly Naples 0.2% respectively. Semliki-forest virus-specific antibodies were detected by plaque reduction neutralization test in 3/351 (0.85%) persons tested. Antibodies against Sandfly Sicilian and Zika viruses were not detected. This study constitutes the first detection of antibodies against Sandfly Naples virus in Kenya. The study has demonstrated the circulation of the selected arboviruses in the two sites amongst human population. These findings will improve our understanding of impact of Arboviruses on public health in the regions so that preventive actions and awareness among clinicians in patient' recognition and management can be enhanced.

918

ASSESSING HEPATITIS AND VACCINES IN A RESOURCE-LIMITED SETTING: THE PERSPECTIVES IN PREGNANCY AND MATERNAL DEATH

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Acute viral hepatitis globally now seems to be a major cause of illness and of death in the developing world and disproportionate cause of deaths among pregnant women. Although, there are accounts of hepatitis vaccine trials that have shown candidate vaccines to be effective and well-tolerated, they are yet to be made available in sufficient quantities to susceptible populations. Verbal interviews and epidemiologic evidence were adopted on the pregnant women visiting the clinics of 2 rural maternity homes/centres in Isseke and Ihiala (both in Anambra State, South East Nigeria). Community survey showed exposure to hepatitis A virus during childhood. Hepatitis C is rare. Hepatitis B is endemic to the rural community, but the incidence of infection and illness are low. Hepatitis E remains the most likely cause of the pregnant women hepatitis-like illnesses. In this context, 17 deaths in women of reproductive age (during pregnancy or within 42 days postpartum) were recorded during July 2012 - February 2013 from both maternal homes. Verbal autopsies identified hepatitis, jaundice and hepatic failure as the primary cause of death in 12 (70.6%) of the 17 pregnancy-related deaths. In conclusion, some data suggests about 10% of deaths observed in pregnancy and in women of reproductive age could be linked to hepatitis. It is then pertinent to demonstrate the effectiveness and safety of the vaccines in the rural settings. To avoid a substantial portion of preventable deaths in these resource-limited settings, judicious and timely implementation of these vaccine interventions would be beneficial.

919

CASE REPORTS AND SEARCH-BASED SURVEILLANCE REVEAL PREDICTABLE, STRONGLY IMMUNIZING DYNAMICS OF HAND, FOOT AND MOUTH DISEASE (HFMD) IN SOUTHEAST ASIA

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Hand Foot and Mouth Disease (HFMD), caused by enteroviruses (primarily EV71, CA16) is a major and increasing cause of serious disease in Southeast Asia. Recently, there has been an alarming increase in the number of patients and an increase in the number of cases complicated by central nervous system and cardiopulmonary involvement and deaths in young children in countries across Southeast Asia in particular. However, the violent epidemic dynamics of these pathogens, and hence the prospects for control with vaccination, are ill understood. We address this problem with a synthesis of public health and web search surveillance time series using a novel platform for epidemiological inference. First, we use lab-confirmed cases from Japan to demonstrate considerable predictability (1-4 years) for EV71 and CA16 epidemics; the associated results indicate strongly immunizing dynamics for both pathogens. Lab-confirmed reports are more sparse for other countries. We address this with Google Trends searches, calibrated first against the Japanese surveillance data; the Google data successfully capture the dynamics of the disease, as the sum of EV71 and CA16. This allows us to extend the analysis more broadly, dissecting the signal of the two pathogens in other countries in the region. Results indicate a relatively optimistic scenario for vaccination, since these infection dynamics reflect sterilizing immunity and relatively moderate transmissibility. More broadly, this synthesis of traditional and novel surveillance with accessible inference provides a powerful tool for clarifying the dynamics of emerging infections.

920

CO-CIRCULATION OF *ORTHOBUNYAVIRUSES* INCLUDING NGARI VIRUS AMONG VECTORS IN ARID AND SEMI-ARID ZONES, KENYA

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Orthobunyaviruses are mosquito-borne viruses that have been associated with febrile illness and hemorrhagic fever in Kenya and elsewhere in Africa. Ngari virus was linked to 27% of the hemorrhagic fever cases during an outbreak of Rift Valley Fever (RVF) in Kenya and Somalia in 1997/98. We have continued to conduct vector-based surveillance to monitor Arbovirus circulation in general and looking out for co-circulation of *Orthobunyaviruses* that would lead to the reassortment and subsequent emergence of viruses like Ngari Hemorrhagic Virus (NRIV). Mosquitoes and ticks sampled between 2009-2010 in Kenya have been screened for Arboviruses by cell culture isolation and identified by RT-PCR. Eleven *Orthobunyavirus* isolates were further analyzed by RT-PCR and sequencing. Isolates of Bunyamwera, Pongola and Illesha were detected and interestingly, four isolates from ticks; *Amblyomma gemma, Rhipicephalus pulchellus* and mosquitoes; *Anopheles gambiae*, *Aedes macintoshi* were identified as Ngari virus sampled from Isiolo, Garissa and Tana-Delta. Other *orthobunyaviruses* were isolated from *Anopheles funestus, Mansonia african* and *Mansonia uniformis* from Magadi and Baringo. It is also noteworthy that we are yet to detect Batai virus, whose M segment constitutes Ngari virus. It is evident that important *Orthobunyaviruses,* including Ngari virus, an emerging hemorrhagic fever virus in E. Africa are circulating widely in parts of Kenya with potential for spread and possibly reassortment through co-infections and movement of animals with ticks. These viruses should be included as differentials in HF diagnosis in the region.

921

RAPID POINT-OF-CARE DETECTION OF SEVEN HEMORRHAGIC FEVER VIRUSES USING REVERSE TRANSCRIPTION RECOMBINASE POLYMERASE AMPLIFICATION ASSAY - TIME TO REACT FAST TO OUTBREAKS

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The most widespread method used for the detection of nucleic acids of etiological agents in clinical samples is real time polymerase chain reaction. During outbreak, clinical samples are sent long distances to the laboratory for analysis, hence real time cyclers are not suitable for point-of-care diagnostics because it is heavy, big, expensive, complex, and must be managed by qualified staff. In addition, test run time is between 60-90 minutes. This study describes the development of real time reverse transcription recombinase polymerase amplification (RT-RPA) assays for the detection of Rift Valley fever virus, Ebola virus, Sudan virus, Bundibugiyo virus, Marburg virus, Dengue Virus, and Yellow fever virus. Quantitative RNA molecular standards were generated and used to determine RT-

RPA assays sensitivity. The analytical sensitivities of RT-RPA assays ranged from 16 to 3778 RNA molecules detected (probit analysis). RT-RPA was performed at 42°C and yielded results after 2 to 15 minutes. The assays showed neither cross-detection of any of the target genomes of the panel nor of the human genome. To test the possibility of using RT-RPA assays for outbreak investigations, a mobile RT-RPA unit was operated in Kedougou, in Senegal. A magnetic-bead based total nucleic acid extraction method was combined with RT-RPA on a portable instrument (tubescanner). Dried pellet RT-RPA reagents and dried oligonucleotides were used. The whole RT-RPA panel was successfully used to detect viral RNA extracted from gamma-irradiation inactivated lyophilised virus supernatants. All operations were carried out at an ambient temperature of 38°C with auxiliary electricity tapped from a vehicle. In conclusion, the developed assay protocol and mobile setup performed well and should be useful for rapid highly sensitive and specific detection of the haemorrhagic fever viruses in outbreak investigations.

922

HIGH RIFT VALLEY FEVER SERO-PREVALENCE IN PEOPLE DURING INTER-EPIDEMIC PERIOD IN THE KILOMBERO VALLEY, TANZANIA

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Rift Valley fever (RVF) occurs in outbreaks in cycles of 5-15 years in the Eastern Africa region and the Horn of Africa, following unusual high rainfall that leads to sustained flooding. In recent years, evidence of RVF transmission during inter-epidemic periods (IEP) in Africa has been reported. The IEP transmissions generally pass undetected in absence of surveillance in mammalian hosts. We studied presence of and the determinants for IEP RVF transmission among humans in an area experiencing annual flooding in southern Tanzania. A sero-survey was conducted among members (n=606) of randomly selected households in 6 villages where a previous study in livestock indicated hotspots of RVF transmission, approximately 5 years after the 2006/07 RVF outbreak in Tanzania. The exposure status to RVF virus (RVFV) was determined using commercial ELISA kits, detecting IgM and IgG antibodies in serum. Information on risk factors for exposure was obtained through structured interviews with the participants. The risk factors were examined by fitting a logistic regression model with villages as random effects where a p-value ≤0.05 was considered statistically significant. An overall seroprevalence of 11.7% (95% CI 9.2-14.5) was recorded with increased exposure in older individuals (OR 1.03; 95% CI 1.01-1.04), in people who milked animals (OR 2.19; 95% CI 1.23-3.91), and individuals eating raw meat (OR 4.17; 95% CI 1.18-14.66). Keeping livestock was not associated with individuals' seropositivity but households keeping livestock had high chances of having at least one member exposed (OR 2.8; 95% CI 1.27-6.17). RVFV IgM antibodies were detected in 4.4% (95% CI 2.9-6.4). Sex, slaughtering, eating meat from dead animal, drinking raw milk, help animal birthing, net use and disposal of aborted fetus were not associated with seropositivity. These preliminary findings indicate IEP transmission with age and interaction with livestock being important risk factors. The high exposure in older individuals and demonstration of IgM antibodies implies a continuous exposure of the population.

923

THE INTERACTOME OF SCHIZONT EGRESS ANTIGEN-1, A NOVEL VACCINE CANDIDATE FOR *FALCIPARUM* MALARIA

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Schizont egress is a complex and tightly regulated process that requires coordination of calcium-signaling, phosphorylation, and proteolysis leading to processing of both parasite and host RBC proteins. Central events include activation of PfPKG, release of PfSUB1 into the parasitophorous vacuole, proteolytic processing/activation of PfSERA5 by PfSUB1, and phosphorylation of unknown substrates by PfCDPK5. We discovered PfSEA-1 using a differential screening approach with plasma from children who were resistant or susceptible to *falciparum* malaria. Antibodies to the immunorelevant region of PfSEA-1 (rPfSEA-1A, aa 810-1083) predict resistance to severe disease in two yr old children, block schizont egress from infected RBC *in vitro,* and vaccination with rPbSEA-1A protects mice from *Plasmodium berghei* ANKA challenge. Moreover, PfSEA-1 localizes to the inner RBC leaflet consistent with a role in remodeling the RBC cytoskeleton prior to rupture. We have explored the protein-protein interactions of PfSEA-1 by screening a yeast two-hybrid Pf3D7 library with rPfSEA-1A as bait followed by confirmation with immuno-precipitation/mass spectroscopy. We have identified 15 interacting proteins including 5 putative substrates and 3 confirmed substrates (PfSERA5, PfRAP-1, and PfRhopH3) of PfSUB1, a protease critical for egress. In *in vitro* phosphorylation assays, we have demonstrated that rPfCDPK5 phosphorylates rPfSEA-1A. Together, these data support the role of PfSEA-1 in the complex schizont egress signaling pathway and suggest additional targets to augment the vaccine efficacy of rPfSEA-1A.

924

MULTIPLE PATHWAYS OF MOSQUITO MIDGUT INVASION BY *PLASMODIUM* OOKINETES

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Plasmodium ookinete invasion of the mosquito midgut is a crucial step of the parasite life cycle. However, the molecular basis for this process is poorly understood. Previous studies from our laboratory showed that the Salivary gland and Midgut peptide 1 (SM1) binds to the lumenal surface of the mosquito midgut epithelium and that this binding results in inhibition of ookinete midgut invasion. Moreover, we found that SM1 is a mimotope of *Plasmodium* surface enolase. Recently, we have shown that both SM1 and ookinete surface enolase bind to a protein on the lumenal surface of the mosquito midgut termed Enolase-Binding Protein or EBP. Based on these and other observations, we hypothesize that ookinete midgut invasion involves the interaction of ookinete enolase and EBP. Cloning of *P. berghei* ANKA 2.34 parasites led to isolation of clones sensitive to SM1 inhibition and clones resistant to SM1. Midgut invasion by ookinetes of SM1-sensitive parasites, but not of the resistant, was blocked by anti-*Anopheles gambiae EBP* (AgEBP) antibodies or by AgEBP gene knockdown. In a separate set of experiments we determined that a peptide from the original phage display screen, Midgut Peptide 2 (MP2), blocks midgut invasion by both SM1-resistant and SM1-sensitive parasites. Moreover, unlike SM1, MP2 also efficiently inhibited midgut invasion by *P. falciparum* ookinetes, suggesting that MP2 binds to a "universal receptor"

required for midgut invasion by ookinetes from most or all *Plasmodium* species. Together these results provide the first evidence that *Plasmodium* ookinetes invade the mosquito midgut epithelium by more than one pathway, as is the case for blood stage merozoites. Characterization of the pathways of *Plasmodium* midgut invasion may lead to new transmission-blocking strategies.

925

CATCHING *PLASMODIUM FALCIPARUM* BETWEEN A ROCK AND A HARD PLACE: SUPPRESSING ANTI-MALARIAL DRUG RESISTANCE WITH AN EVOLUTIONARY TRAP

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Drug resistance emerges in an ecological context where fitness costs restrict the diversity of escape pathways. These pathways are targets for drug discovery. Blocking the most fit resistance pathways by combining wild-type and mutant-type inhibitors may prevent the emergence of competitively viable resistance. We tested this hypothesis with malaria parasites derived by *in vitro* selection with dihydroorotate dehydrogenase (PfDHODH) inhibitors. We selected *Plasmodium falciparum* lines resistant to structurally unrelated PfDHODH inhibitors (Genz-666136 and DSM74), and found a point mutation in PfDHODH (E182D). We discovered a compound more potent against E182D than wild-type parasites (IDI-6273). Mutant-specific drugs allow us to set a evolutionary trap: if resistance to one compound greatly increases sensitivity to another, then parasites that become resistant to one compound will be battered by the second. Selection of the E182D mutant with IDI-6273 gave a second nucleotide mutation in pfdhodh. This mutation gave a reversion to the wild-type protein sequence and drug-sensitivities. Combination selection with a wild-type PfDHODH inhibitor and IDI-6273, the mutant-selective inhibitor, did not yield resistant parasites in eighty days - ample time for every position in the genome to be mutated at least once. Recombinant wild-type and E182D PfDHODH protein mirrored the drug sensitivities seen in whole-cell assays, and also demonstrated that the E182D mutant has little effect on substrate affinity. This may explain why E182D is a favored mutation pathway, as other nearby mutations have larger detrimental effects on enzyme activity and thus overall fitness. We believe that evolutionary fitness constraints allow few pathways to resistance, and these pathways can be anticipated and preemptively blocked. The combination of well-chosen anti-malarial agents active against sensitive and resistant parasites effectively kills parasites in the short-term, and in the long-term, can help shape parasite evolution away from the development of drug resistance.

926

A NEW CLASS OF ANTIMALARIAL COMPOUNDS TARGETING THE NON-MEVALONATE PATHWAY ENZYME ISPD

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Antimicrobial drug resistance is an urgent problem in control and treatment of many of the world's most serious infections, including *Plasmodium falciparum* malaria and tuberculosis. Because the non-mevalonate pathway of isoprenoid biosynthesis is essential in eubacteria, mycobacteria, and *P. falciparum*, but not present in humans, there is great interest in targeting the enzymes of this pathway for antimicrobial and antiparasitic drug development. Our previous studies indicated that the enzyme IspD (E.C. 2.7.7.60; methylerythritol cytidyltransferase) was a point of particular metabolic control of isoprenoid biosynthesis in *P. falciparum* malaria parasites. We aimed to develop new lead compounds for antimalarial drug development targeting Pf-IspD. Our strategy relies on chemoinformatic modeling and in silico compound selection prior to physical screening of compounds against purified recombinant enzyme. We optimized high-throughput, reproducible assay conditions for Pf-IspD. We screened 5,000 diverse compounds, which were selected on the basis of predicted activity and promising ADMET properties from the large compound library available at Biofocus. From the most active of our initial hits, a series of compounds have been developed that inhibit the growth of cultured *P. falciparum* (IC_{50} of approximately 1 μM) with encouraging structure activity relationships. Metabolic analysis of compound-treated malaria cultures demonstrates inhibition of isoprenoid biosynthesis, confirming mechanism of action. Initial cellular toxicity studies demonstrate limited toxicity to human cell lines. Ongoing studies include medicinal chemistry efforts to improve compound activity and rescreening of additional Biofocus compounds to identify further chemical series. Our studies demonstrate the feasibility of targeting Pf-IspD for antimalarial development and have identified a new, promising class of antimalarial compounds.

927

GENOMIC SCALE EXPERIMENTAL GENETICS TOOLS FOR *PLASMODIUM BERGHEI*

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The high AT-content of *Plasmodium berghei* genomic DNA precludes the production of large and complex genetic modification vectors by conventional restriction/ligation cloning into high-copy, circular plasmids. Two recent developments have converged to help us overcome this problem. Firstly, using a low copy, linear plasmid we generated the first high integrity genomic DNA library (PbG), which covers >90% of *P. berghei* genes on large genomic inserts of 9 kb on average. An arrayed library of end-sequenced PbG clones is now searchable online and clones are available as a free resource. Secondly, we developed a molecular tool kit and a set of protocols to modify PbG inserts using the Red/ET recombinase system from lambda phage, as reported previously. I will illustrate how recombinase-mediated engineering ("recombineering") of PbG clones can be used by individual laboratories to generate different genetic modification constructs, ranging from simple knock out and tagging vectors to more complex conditional alleles that use the FLP/frt system, as well as allelic exchange and complementation vectors for large genes that could not have been made using conventional techniques. Recombineered vectors have a much improved recombination frequency thanks to their long homology arms and all methods used for their production are sufficiently robust to scale to a 96-well format. Recognising these unique advantages, the Malaria Programme at the Wellcome Trust Sanger Institute has initiated the PlasmoGEM project, which aims to produce and distribute genome-wide, free resources of *Plasmodium* genetic modification vectors (http://plasmogem.sanger.ac.uk). We initially aim to generate and share the largest possible set of vectors for the targeted deletion and c-terminal epitope tagging of *P. berghei* genes. Vectors will be ready for marker recycling by negative selection and will feature gene-specific barcodes to serve as signature tags in genetic screens. The current status of the resource will be presented, along with its applications to genetic screens that could encompass the entire *Plasmodium berghei* genome.

928

PLASMODIUM GAMETOCYTES DENSITIES IN BURKINA FASO, IMPLICATIONS FOR IMPLEMENTATION OF TRANSMISSION-BLOCKING INTERVENTIONS

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Plasmodium gametocytes, the sexual-stage parasite in human blood are the transmissible forms to the *Anopheles* mosquito vectors, and thus mediate the onward transmission of the malaria. Breaking the human to mosquito transmission is a new promising concept of malaria control. To date, some transmission-blocking vaccine (TBV) candidates and drug molecules have been showed to limit the transmission of *Plasmodium*. Such strategies require good knowledge of the transmissible parasite stages biology in the field. In this purpose, a four year longitudinal *Plasmodium* prospection was investigated in the young children population of rural endemic area in Burkina Faso (West Africa), for *Plasmodium* infection. Malaria parasitogical tests were performed for any "healthy" child, and parasite density was quantified for each infected case. A total of 19,500 microscopic tests performed in 2000 children population showed *Plasmodium* infection in the children population at each month of the year. Analysis revealed more than 40% and 8% of asexual parasite and gametocyte infections respectively, per year. For 1599 gametocyte infections, ranged from 8 to 9256 gametocytes/µl of blood, an average of 55.20 gametocytes/µl (95% CI [45.79; 64.61]) was found. Regarding these densities the efficacy of two TBVs candidates (Pfs25 and Pfs230), as shown in laboratory in Burkina Faso, might be sufficient to limit *Plasmodium* transmission. Furthermore, following the temporal variation, the lower gametocyte density (33.10 gametocytes/µl 95% CI [22.72; 43.48]) observed from February to April suggest this period to be appropriated for any transmission blocking interventions.

929

EVALUATION OF TRANSMISSION RESERVOIR AND FIELD FEEDING ASSAYS TO INFORM TRANSMISSION BLOCKING VACCINE FIELD TRIAL DESIGN

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A transmission blocking vaccine (TBV) could be an integral part of malaria elimination and eradication. In preparation for a Phase 1 trial to test a Pfs25-based TBV malaria vaccine candidate in the target population, we surveyed gametocyte carriage rates by microscopy reading of thick smears and by quantitative PCR in Bancoumana, Mali. Starting jn June 2011, a total of 500 volunteers, from 3 months to 50 years of age, were recruited for monthly surveys of asexual and sexual parasite carriage. The carriage rates were higher among volunteers living closer to river and rice fields. Although the carriage rates were higher (12%) in age groups of 5-17 years old, typically 9% of adults older than 18 years of age carry gametocytes during the high transmission season. These adults are infectious to mosquitoes, as shown by various artificial feeding methods including Direct Skin Feeds (DSF) and Direct Membrane Feeds (DMF). Baseline mosquito infectivity was higher by DSF, followed by DMF with serum replacement. Smear reading by well-trained microscopists proved to be a very effective way to identify gametocyte carriers infective to mosquitoes. Based on these data, a Phase 1 study has been designed and planned to evaluate safety, immunogenicity, and transmission blocking activity induced by the Pfs25-EPA/Alhydrogel vaccine candidate product in adults in Bancoumana, Mali.

930

ANTIBODIES AGAINST A YEAST EXPRESSED PFS230 PROTEIN PRODUCED BY SCALABLE BIOPROCESSES BLOCK MOSQUITO TRANSMISSION OF MALARIA PARASITES AND FUNCTION TOGETHER WITH ANTIBODIES AGAINST PFS25

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Development of a *Plasmodium falciparum* transmission blocking vaccine (TBV) has the potential to markedly impact malaria control efforts. Pfs25-EPA, a leading TBV candidate is a chemically conjugated nanoparticle adsorbed on Alhydrogel®. In a phase 1 safety and immunogenicity trial, Pfs25-EPA/Alhydrogel® appeared safe and induced antibodies that partially blocked mosquito infectivity using an *ex vivo* feeding assay (SMFA). However, the level of reduction in transmission blocking activity appears insufficient in its current formulation. Efforts to evaluate another promising TBV candidate in the clinic, identified as the 230 kDa sexual stage protein Pfs230, have been thwarted for nearly a quarter of a century due to an inability to produce clinical grade protein containing the unique 6 cysteine-rich motif. Here, we report the scalable production of a recombinant subdomain of Pfs230, specifically Pfs230 domain 1 (D1) using a modified *Pichia pastoris* host. The secreted and purified recombinant Pfs230D1 protein has been fully characterized biochemically and biophysically. Antibodies against Pfs230D1 alone blocked transmission of malaria parasites and also had blocking effects when used in the presence of anti-Pfs25 antibodies by SMFA. Pfs230D1 and Pfs25 formulated in GLA-SE, the synthetic Toll-like receptor 4 agonist Glucopyranosyl Lipid Adjuvant in Stable Emulsion induced antibodies in *Aotus* monkeys that blocked mosquito transmission of malaria. The 6 cysteine-rich domain of Pfs230D1 is completely conserved among 200 parasite isolates. The combination of Pfs230D1 and Pfs25 in the form of chemically conjugated nanoparticles is a promising next step toward development of a vaccine that blocks malaria transmission.

931

RECOMBINANT PVS48/45 ANTIGEN EXPRESSED IN *E. COLI* GENERATES ANTIBODIES THAT BLOCK MALARIA THE TRANSMISSION IN *ANOPHELES ALBIMANUS* MOSQUITOES

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Transmission of malaria parasites from humans to *Anopheles* mosquitoes can be inhibited by specific antibodies to surface gametocytes proteins elicited during human malaria infection, these proteins are therefore considered to have potential for vaccine development. *Plasmodium vivax* Pvs48/45 is considered a promising gametocyte membrane surface antigen due to its potential functional role during parasite fertilization. The protein belongs to the 6-cy domain family characterized by its structural complexity what makes difficult to obtain high yields of soluble and properly folded protein when expressed as recombinant product. We

describe the expression of Pvs48/45 in *E. coli* and its biochemical and immunological characterization. The ~54kDa affinity-purified protein was reactive with sera (n= 57) of individuals from malaria endemic areas of Colombia and induced high ELISA antibody titers in mice and in Aotus monkeys when injected emulsified in Montanide ISA-51(. Antibodies from both mice and primates recognized the native parasite protein by Western blot and IFA (mean titer= 1:10,240) and efficiently blocked (>90%) the infection of *Anopheles albimanus* mosquitoes in membrane feeding assays. These results indicate that rPvs48/45 protein expressed in *E. coli* has the proper conformation able to induce antibodies that block sporozoite fertilization in the mosquito vector and therefore could be used as potential target to design a TB vaccine against *P. vivax*. Ongoing studies on the optimization and further protein characterization as well as immunogenicity and TB efficacy studies in primates will be presented.

932

A PHASE IA CLINICAL TRIAL OF A BLOOD-STAGE *PLASMODIUM VIVAX* VACCINE

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Plasmodium vivax is the most geographically wide-spread malaria and is increasingly recognised as a significant cause of morbidity and economic loss, as well as occasional mortality. Control of *P. vivax* is challenging, with re-emergence in areas where it has previously been eliminated. Little progress has been made in development of a *P. vivax* vaccine with only two *P. vivax* antigens reaching Phase Ia clinical trials. Here we report on a Phase Ia clinical trial in Oxford using recombinant simian adenovirus ChAd63 and modified vaccinia virus Ankara (MVA) encoding the *P. vivax* antigen PvDBP (Duffy-binding protein region II) in a heterologous prime-boost regimen. Invasion of reticulocytes by *P. vivax* requires interaction between the parasite ligand Duffy-binding protein and its host receptor, the Duffy antigen receptor for chemokines (DARC). Unlike *P. falciparum* which can utilise multiple redundant pathways for erythrocyte invasion, the interaction between PvDBP and DARC is vital for *P. vivax*, making PvDBP a very promising antigen for vaccine development. Pre-clinical studies have shown that ChAd63 and MVA expressing PvDBP induce strong antibody immunogenicity and functional activity to block binding of PvDBP to DARC *in vitro*. This Phase Ia study of ChAd63-MVA PvDBP involves vaccinating 24 healthy volunteers in a dose escalation study. The dosing schedule involves ChAd63 PvDBP at 5 x 109 vp in the first four volunteers; ChAd63 PvDBP at 5 x 1010 vp in the next four volunteers; then two groups who will receive both ChAd63 PvDBP (5 x 1010 vp) followed by MVA PvDBP eight weeks later (at doses of 1 x 108 pfu - 2 x 108 pfu). Safety is the primary objective and safety reviews are undertaken throughout the study, particularly prior to dose escalation. The secondary objective is humoral and cellular immunogenicity which will be assessed using assays including PvDBP IFN-γ T cell ELISPOT, B cell ELISPOT, PvDBP IgG antibody ELISA and functional antibody analysis. This clinical trial is the first to be undertaken for a blood-stage antigen targeting *P. vivax* and will provide valuable insight into the utility of human vaccination against this well-described antigen.

933

IDENTIFYING CRYPTIC PROTECTIVE EPITOPES, GENERATING FULLY HUMAN MONOCLONAL ANTIBODIES AND APPLYING REVERSE VACCINOLOGY TO PROMOTE SUCCESSFUL MALARIA VACCINE DEVELOPMENT

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Several published studies indicate that in animal models of malaria, passive immunization with monoclonal antibodies targeting protective peptides contained within the sequence of leading malaria vaccine candidate antigens such as the circumsporozoite protein (CSP) provides much superior protection relative to active immunization with recombinant protein that includes the protective peptide, or with the protective peptide itself. While active immunization with recombinant protein or peptide induces overall antigen-specific antibody titers of equivalent magnitude to passive immunization with a monoclonal, as evidenced by sporozoite IFA or CSP ELISA, incubation with the protective epitope to absorb out peptide-specific activity does not appreciably affect the IFA titer of the actively-induced sera, indicating the presence of minimal antibody targeting the protective peptide. These observations suggest that evolutionary molding of protein architecture in *Plasmodium* due to continuous exposure to host immunity has resulted in antigens that are fundamentally poorly immunogenic for protective responses. In this presentation, these data will be reviewed and approaches will be proposed that could lead to effective passive immunization against malaria, or to the design of immunogens that, although potentially straying from native amino acid sequence, nevertheless induce protective responses in vaccine recipients. Key to these approaches is an emerging technology developed at the Naval Medical Research Center, US Military Malaria Vaccine Program, based on humanized mice, that can be used to rapidly generate fully human (and thus non-allergenic) monoclonal antibodies targeting malaria proteins. While manufacturing processes still require refinement, these reagents, likely both safe and amenable to repeated administration without loss of effectiveness, may gain importance for malaria prevention and treatment, and for regional campaigns for malaria elimination.

934

EFFECTS OF EARLY PARASITIC INFECTIONS ON CHILD GROWTH IN COASTAL KENYA

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Children in resource-limited tropical communities are heavily burdened by parasitic diseases that lead to high rates of morbidity and mortality. Few studies have examined at what age parasitic infections begin and what effects they have on early growth. Our objective was to determine the incidence and prevalence of parasitic infections (malaria, urinary schistosomiasis, soil-transmitted helminthes (STH), and lymphatic filariasis) in the first two years of life and examine their effects on growth. A 2005-2010 child cohort in Coastal Kenya was followed at birth and every 6 months until age 2 years. At each visit height, weight, and head circumference were measured and blood, stool, and urine were collected. Specimens were tested for presence of infection as follows: blood smear and PCR for malaria, Og4c3 antigen ELISA for filaria, Ritchie stool examination for STH, and SWAP IgG4 ELISA and urine filtration for *S. haematobium*. We computed descriptive statistics to estimate the infection rates. Mixed linear models were developed to determine the association

of each type of infection with growth variables.Of 182 children, 20 (11%) had at least one parasitic infection at 6 months, 32 (18%) at 12 months, 60 (33%) at 18 months, and 62 (34%) at 24 months. 56 (31%) of children had one infection and 14 (7.7%) were polyparasitized by the age of 2 years. All infections were documented as early as 6 months of age, except *Ascaris*. Infection prevalence increased with age. Malaria infection showed a statistically significant decrease in head circumference z score at 18 months (p=0.01). By 24 months, weight, height and head circumference z scores were decreased as compared to the uninfected group (p=0.05, 0.02, 0.01, respectively).Children with schistosomiasis during the first two years of life had a decrease in height z score (p=0.05). Children infected with STH had a significant decrease in head circumference z score by 24 months (p=0.05). *Strongyloides* infection was associated with a decrease in weight z score by 24 months (p=0.04). Parasitic infections begin early in infancy and increase in incidence in the first two years of life in Coastal Kenya. Malaria, schistosomiasis, and STH infections were associated with significant decreases in growth parameters by 24 months of age.

935

QUALITY OF CASE MANAGEMENT OF CHILDHOOD ILLNESSES PROVIDED BY HEALTH EXTENSION WORKERS IN ETHIOPIA

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Ethiopia is scaling-up integrated community case management of common childhood illnesses (iCCM) in most regions of the country. Antibiotic therapy for childhood pneumonia and zinc for treatment of diarrhea have been added to the pre-existing management of malaria, diarrhea (with oral rehydration solution only) and malnutrition. We surveyed Health Extension Workers (HEWs) to assess the strength of iCCM implementation and the quality of care provided to sick children. This study was the first to evaluate the scale-up of iCCM in Ethiopia and the first rigorous assessment of quality of care provided by HEWs. It also adds to a limited evidence base on the quality of iCCM services provided by community-based health workers in sub-Saharan Africa. We conducted a cross-sectional survey in random samples of health posts in iCCM intervention and comparison areas in two zones of Oromia region. A total of 201 HEWs in 149 health posts were surveyed and 257 sick children were included. Data collectors trained in iCCM observed HEWs' consultations with sick children, carried out 'gold standard' re-examinations of children, conducted caretaker exit interviews, inspected iCCM commodities and patient registers and interviewed HEWs. Indicators of program strength, including availability of commodities and supervision, were significantly higher in intervention areas. HEWs in intervention areas provided correct treatment and/or referral for 64% of children with major iCCM illnesses (pneumonia, diarrhea, malaria, malnutrition and measles). The proportions of children correctly managed for pneumonia, diarrhea and malnutrition were 72%, 79% and 59%, respectively. Only 6% of children received an antibiotic when it was not indicated and no children received unnecessary antimalarials. However, only 34% (13/38) of children with severe illness were correctly managed and 54% (34/63) of children needing referral to a higher-level health facility were referred. Utilization of HEW case management services was low, with an average of only 16 sick child consultations per health post in the previous month in intervention areas. Implementation of the iCCM program in Jimma and West Hararghe zones was very strong and HEWs are generally providing high-quality case management services. However, for the program to have a significant impact on child mortality, management of severe illnesses and utilization of HEW services must be improved.

936

MATERNAL INFECTIONS AND ANEMIA IN INFANCY AND EARLY CHILDHOOD

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Iron deficiency (ID) is common in pregnant women and children worldwide. When ID occurs in infancy, neurological development can be impaired. Through transplacental iron transfer, infants acquire iron stores *in utero*. Factors such as chronic inflammation or profound ID in mothers can impair iron transport, thus increasing a child's ID risk. To assess risk factors for anemia in infancy and childhood, we studied offspring of Kenyan women living in nearby rural (N=303) and urban (N=418) areas, assessing hemoglobin (Hb) levels at birth and at 6, 10, and 14 weeks of age, and every 3 months thereafter until age 3. Forty-two percent of mothers residing in the rural area were anemic (Hb<9 gm/dl) at delivery, as compared to 4.7% of urban women; despite this difference, newborns in the rural and urban sites had similar mean Hb levels and frequencies of anemia (Hb<12.5 gm/dl,16.8% and 17.9%, respectively). Mean Hb in offspring remained similar between sites until after 10 weeks of age, when mean Hb diverged; children in the rural population had a 2-3 gm/dL lower mean Hb when compared to urban children, and the difference persisted until age 2 years. This finding could not be explained by sex, variation in feeding practices, rates of weight gain or maternal mean Hb level at the sites. Although maternal age and maternal BMI were associated with low mean Hb levels in offspring, these factors also did not explain differences in mean Hb. However, we found that maternal helminth and malaria infections during pregnancy had been documented in over 60% of the rural women, while in urban women the frequency was <10%. Whether these women developed anemia of chronic inflammation and/or ID is under investigation. We suggest that women with chronic parasitic infections develop anemia of chronic inflammation, impairing transplacental transport of iron during pregnancy. This could result in inadequate iron stores in offspring and insufficient support of erythropoiesis at 10 weeks of age. The importance of treating chronic parasitic infections during pregnancy should be emphasized.

937

IMPACT OF AN INTENSIVE PERINATAL HANDWASHING PROMOTION INTERVENTION ON MATERNAL HANDWASHING BEHAVIOR IN THE NEONATAL PERIOD: FINDINGS FROM A RANDOMIZED CONTROLLED TRIAL IN RURAL BANGLADESH

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One-quarter of neonatal deaths are attributed to infections, including sepsis and pneumonia. Maternal handwashing with soap prevents pneumonia among young children, and may prevent neonatal sepsis. We examined the impact of an intensive handwashing promotion implemented in the perinatal period on the handwashing behavior of mothers of neonates. We conducted a randomized controlled trial enrolling pregnant women at 28-32 weeks gestation in Matlab, Bangladesh. After collecting baseline data, we randomized participants to intensive handwashing promotion or control. Both study arms received maternal and neonatal care counseling and a clean delivery kit. In 1-2 antenatal visits, and 2 post-natal visits, we used a participatory approach to motivate maternal handwashing with soap, promoting handwashing as a nurturing behavior, among the intervention arm; soap and water containers were provided for handwashing in the neonate's sleeping room,

the courtyard, and outside the latrine. In both study arms, we observed presence of soap and water at handwashing places during Weeks 1, 2, and 4 after birth. Using data from 3-hour structured observations in Week 4, we estimated intervention impact on maternal handwashing overall and at possible times of pathogen transmission. In total, 125 women were randomized to intervention and 124 to control. During the neonatal period, soap and water were present more frequently at a handwashing place in intervention homes than controls: Week 1 (84% vs. 45%); Week 2 (69% vs. 35%); Week 4 (69% vs. 27%). In Week 4, observed handwashing overall was 40% higher among intervention mothers than controls (p<.001). Although soap was observed at a minority of pathogen transmission times in both intervention (8%) and control (2%) groups, intervention mothers washed hands with soap 4.1 times as frequently as controls (p<.0001). Intensive promotion of handwashing with soap, and provision of needed materials, in the perinatal period resulted in increased availability of soap and water at designated handwashing places, and a four-fold increase in maternal handwashing with soap. Although handwashing with soap was infrequent at possible times of pathogen transmission, the overall increase in handwashing with soap may be sufficient to reduce neonatal infections.

938

HOME-BASED MANAGEMENT OF MALARIA, DIARRHEA AND ACUTE RESPIRATORY ILLNESS IN CHILDREN: THE EXPERIENCE IN SENEGAL

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In 2008, the Senegal National Malaria Control Program (NMCP) introduced home-based management of malaria (PECADOM), with excellent results. Evaluations of the program recommended including diarrhea and acute respiratory illness (ARI) management in the package of interventions to increase impact on all cause under five mortality. The NMCP and partners conducted a pilot of PECADOM integrating diarrhea and ARI management in 87 villages in five districts. A volunteer home care provider (DSDOM) was selected from each of the communities and participated in four days of classroom training and a 15 day practicum. After training, an installation ceremony was held for each DSDOM, and each received a medical supply box with a timer, rapid diagnostic tests (RDT), artemisinin-based combination therapy (ACT), oral rehydration salts, zinc, and co-trimoxazole. All patients were recorded in a register. Monitoring included supervision, monthly coordination meetings and biannual reviews including all involved in the implementation of integrated PECADOM at all levels. Data were obtained from registers, supervision visits, and the biannual review. Interviews were conducted with members of the community. A total of 87 DSDOMs were trained and installed. During six months, 3,177 children under five years were seen in consultation by DSDOMs. Of these, 13% had fever and received an RDT, 18% had a cough and runny nose, 31% had uncomplicated pneumonia, and 29% had diarrhea. There were 173 confirmed cases of malaria, all of which were reported treated and cured. On average, 10% of cases seen in consultation by DSDOMs were referred to health posts, the majority of which were cases with negative RDT. Communities were actively involved and welcomed the implementation of the strategy in their village. The DSDOMs expressed a sense of purpose and respect in their community. The encouraging results achieved after six months of implementation of the pilot suggest the need to scale up to ensure early and universal access to quality case management of malaria, diarrhea and ARI. Integrated PECADOM is an important tool to ensure equity in access to care and decrease all cause under five mortality.

939

VARIATION IN THE QUALITY AND COST OF TREATMENT FOR CHILDHOOD DIARRHEA, MALARIA AND PNEUMONIA: COMMUNITY AND FACILITY BASED CARE IN RURAL UGANDA

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Diarrhoea, malaria and pneumonia together account for 36% of deaths in children under 5yrs of age, yet facility-based treatment is often inaccessible or inadequate in low income/rural settings. Integrated community case management (iCCM) has been recommended by the WHO/UNICEF as an effective means of tackling the treatment gap through the training and deployment of community health workers (CHWs). The inSCALE study aims to improve the motivation and performance of CHWs and thus increase coverage of appropriately treated children in Uganda and Mozambique. As part of a cross-sectional survey of 6553 randomly sampled households in villages with iCCM CHWs in western Uganda, we identified 3900 households with 6501 children aged 2-59months. 11%, 47% and 24% of study children had recently had episodes of diarrhoea, fever and pneumonia (DFP) respectively. Only 54% of children were given appropriate antibiotics for pneumonia, 47% with fever were given ACTs, rising to 73% in malaria-confirmed cases, and 30% received ORS for diarrhoea. Coverage of ORS+zinc treatment for diarrhoea was low at 9% as was blood testing (RDTs or microscopy) in cases of fever (27%). Relatives who sought care for sick children accessed CHWs in 22% of cases, whilst the majority visited health facilities (63%) the largest proportion of which were private facilities or doctors (38%). However the chances of receiving correct treatment for DFP was lowest in the private sector (23%, 32% and 56% respectively) and highest via CHWs (53%, 71% and 61% respectively) which were also the least expensive option at $0/visit, compared to $2/visit in the private sector. There was a small but consistent trend of reduced access to appropriate treatment in the poorest 40% of households vs. the richest 40% (coverage differences -6%, -6% and -10% for DFP respectively). iCCM has great potential to reduce coverage gaps in the treatment of community-acquired infections at low cost to families. CHWs were accessed in a small number of cases of DFP; this is likely to increase over time with the embedding of the national iCCM policy.

940

OUTBREAK OF SUDDEN DEATHS AMONG CHILDREN LIVING NEAR LYCHEE ORCHARDS IN NORTHERN BANGLADESH

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In June 2012, a cluster of sudden deaths among children aged 2-10 years was reported from northern Bangladesh. In response, a team from the Government of Bangladesh's Institute of Epidemiology, Disease Control and Research and icddr,b conducted an investigation to describe clinical

and exposure histories of cases to generate hypotheses about possible causes of illness; the minimum criterion for classification as a suspected case was presence of convulsion. We reviewed hospital records to identify cases and interviewed family caregivers about the symptoms and exposures the case had. Since many case-households were located adjacent to lychee orchards, we collected information on the types of pesticides used in the area and case-patient exposures to the orchards and lychees. Fourteen children met the case definition; 13 (93%) died and the median time from illness onset to death was 20 hours. For 64% of cases, the illness started with a sudden outcry in the early morning followed within hours by convulsions and unconsciousness. The most common signs were convulsions (100%), unconsciousness (86%), frothy discharge from the mouth (86%), altered mental status (71%) and fever (70%). Four children had mid-dilated or fixed pupils and six had lung crepitations on auscultation. In the 24 hours before illness onset, all of the cases had either visited lychee orchards (n=11) or consumed lychees (n=7) where multiple pesticides including insecticides, fungicides and other chemicals were used at least three times during the preceding two weeks. Eight case-households bordered lychee orchards, and five case-households were located within approximately 100 meters of a lychee orchard. Caregivers reported that many cases peeled lychee fruits with their teeth and ate unwashed lychees. The clinical manifestations and course of illness of cases suggest that this outbreak was due to poisoning, likely from pesticides used in nearby lychee orchards. Close proximity of the case-households to lychee orchards, heavy use of pesticides in the orchards, and the children's exposure to lychee orchards and fruit increased their exposure to agricultural chemicals. Interventions are needed to limit children's exposures to dangerous agricultural chemicals pesticides. In South Asia, repeated outbreaks of child encephalitis during lychee harvesting season have been reported in with undetermined etiology which could be similar to what we observed in Bangladesh.

941

USE OF ANTHROPOGENIC MOSQUITOES FOR SURVEILLANCE OF BLOOD-BORNE PATHOGENS

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Biosurveillance is the process of collecting, analyzing, and interpreting biosphere data in order to provide early detection of biological threats to human health. Obtaining meaningful samples for surveillance, particularly human blood, has limited pathogen detection in many underdeveloped countries. Therefore, we explored the possibility of exploiting the intrinsic host-feeding preferences and behaviors of Anopheline mosquitoes to enhance biosurveillance efforts. Earlier studies have demonstrated the technical feasibility of this approach. Specifically, human papillomavirus was detected in field collected mosquito pools during a metagenomics analysis of the mosquito virome. In addition, highly pathogenic avian influenza virus was detected in engorged mosquitoes from a poultry farm in Thailand by RT-PCR. In this study we aimed to define the limitations of this approach in the laboratory and determine its potential application in the field. We hypothesize that blood-fed mosquitoes can be used in surveillance and discovery of pathogens in underdeveloped countries. To test this, colonized *Anopheles gambiae* mosquitoes were fed on blood containing serial dilutions of chikungunya, influenza A, West Nile, Pirital, and human immunodeficiency-1 viruses. Twelve hours later the contents of the mosquito midguts were pressed onto filter paper to inactivate the virus and preserve the nucleic acids. The limits of viral RNA detection by RT-PCR were between 10 and 100 genome equivalents per microliter of fed virus. Additionally, we could differentiate between human, dog, cow, and sheep blood meals using multiplexed PCR. The samples will be further assessed for detection limits using deep sequencing. In August of 2012 our pathogen surveillance system was evaluated using blood fed *An.* mosquitoes collected at dawn from homes in Bandafassi, Senegal. Preliminary deep sequencing results of the mosquito blood meals revealed the presence of RNA homologous to herpes simplex virus type 2 and several *Plasmodium* species. We have demonstrated that this is a creative solution to overcome the technical and logistical obstacles, such as training local health professionals and maintaining sample cold-chain, for acquiring human blood for pathogen surveillance.

942

BIODISTRIBUTION AND TRAFFICKING OF NANOPARTICLES IN *ANOPHELES GAMBIAE* ADULT AND LARVAL MOSQUITOES

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There is a public health imperative to develop new chemical insecticides with new modes of action for vector control. Emerging resistance to pyrethroids, the principal active ingredients (AIs) in bednets and in indoor residual and space spraying, is of immense concern. We are developing an innovative, flexible and adaptable, molecular mosquitocide (MM) approach to control disease vectors. A MM is comprised of 1) an "active sequence" (AS) dsRNA to induce an RNAi response to silence a targeted mosquito gene and 2) a nanoparticle delivery system that promotes environmental stability of and efficacious delivery of the AS to vector target organs and cells to induce a systemic RNAi response resulting in vector lethality. This approach exploits the explosion of information accrued in mosquito genetics and genomics to provide an almost unlimited number of potential target genes and sequences for RNAi-based MMs. Critical to the development of MMs is selection of optimal nanoparticles for delivery of the ASs. Nanoparticles are produced using state of the art Particle Replication in Non-wetting Templates technology. Using PRINT^{R} Platform technology nanoparticles of defined size, shape, aspect ratio, surface charge and modulus have been fabricated and compared in their biodistribution in adults and larvae following *per os* challenge and intrathoracic injection. Nanoparticles do differ dramatically in their biodistribution and cellular internalization potential depending upon such characteristics as size, aspect ratio, and charge. Studies to determine optimal particle characteristics for delivery of ASs (approximately 400 bp dsRNAs) to selected programmed cell death genes to induce a lethal, systemic RNAi response are in progress. MMs offer exciting potential to expand the armamentarium for vector control beyond the MOAs of conventional chemical insecticides and to potentially provide a flexible platform approach for delivering new and improved ASs to vectors in response to the emergence of resistance.

943

LADIES FIRST: EFFECT OF GENDER ON BITING RISK BY TSETSE FLIES

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There is renewed vigour in efforts to eliminate sleeping sickness (human African trypanosomiasis; HAT) caused by subspecies of *Trypanosoma brucei* transmitted by tsetse flies. A better understanding of risk factors for HAT would contribute to this effort. Towards this goal, we carried out studies in the West Nile region of Uganda to assess how traditional gender roles influence exposure to tsetse. Sixty participants (thirty women and thirty men) from randomly selected households located in historical Gambian HAT foci wore a small global positioning system (GPS) during the course of their usual daily activities. Participants were also interviewed about their daily routines. GPS data were analysed using a geographical information system (GIS) and transcribed interviews were analysed using thematic analysis. Gender-specific tasks related to fetching water and gathering

firewood meant that the number and duration of visits to river banks and forested areas, where tsetse are more abundant, was greater for women than men. Women were also more likely to visit these risk zones during times of day when tsetse are active. Farming activities undertaken by men and women also increased exposure to tsetse, especially during the dry season when planting of crops and herding of livestock is concentrated in riverine areas. Overall, we estimate that women were more than twice as likely to visit tsetse-infested habitats as men. The implications of our results for the transmission and control of HAT are discussed. We conclude that strategies specifically directed at reducing risk of sleeping sickness for women are needed.

944

EXAMINATION OF THE DEVELOPMENTAL NEUROGENETIC BASIS OF SEXUAL DIMORPHISM IN *AEDES AEGYPTI*

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Most animal species exhibit sexually dimorphic behaviors, many of which are linked to reproduction. A number of these behaviors, including blood feeding in female mosquitoes, contribute to the global spread of vector-borne illnesses. However, knowledge concerning the extent of sexual dimorphisms in the structure of the central nervous system, the control of sex-specific behaviors by sexually dimorphic neurons, and the developmental genetic basis for sexually dimorphic behavior is limited in any organism, including mosquitoes. In this investigation, we used custom microarrays to examine global differences in male vs. female gene expression in the developing brain of the dengue and yellow fever vector mosquito *Aedes aegypti.* The array uncovered 2,528 statistically significant differentially expressed genes. Genes upregulated in females were predominantly implicated in proteolytic and metabolic gene ontology (GO) processes. Genes upregulated in males were often associated with metabolic GO terms, including polysaccharide metabolism. Genes which have been implicated in behavior, including feeding, were also dimorphically expressed. A number of differentially expressed genes were also linked to critical developmental signaling pathways, including the Wnt and Hedgehog signal transduction cascades. Genes from these metabolic, behavioral, and developmental pathways were prioritized for whole-mount gene expression analyses in the pupal brain. These expression studies validated the microarray results and allowed for identification of sexually dimorphic regions in the pupal brain. In many cases, dimorphic gene expression localized to the optic lobe. We are now pursuing siRNA-mediated knockdown studies to functionally examine the roles of dimorphically expressed genes. These studies are providing insight into the genetic differences underlying sexually dimorphic neural circuitries and behaviors that promote the spread of disease.

945

COORDINATE REGULATION OF ODORANT RECEPTOR EXPRESSION AND OLFACTORY RECEPTOR NEURON TARGETING IN *AEDES AEGYPTI* BY THE TRANSCRIPTION FACTOR SINGLE-MINDED

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Knowledge of the mechanism that specifies mosquito olfactory receptor neurons (ORNs) to express a particular odorant receptor (OR) from a large OR pool, an important step for odor detection and discrimination, is lacking. Here, we investigate this process in *Aedes aegypti*, the dengue and yellow fever vector mosquito. Studies in other organisms have suggested that the combination and levels of expression of various cis-regulators of transcription in ORNs generates the OR regulatory matrix, a code governing which particular OR gene is expressed and which are repressed in any given ORN. Mosquito OR expression is likely to be regulated in a comparable manner. We have identified eight transcription factors (TFs) which are expressed in the developing antennae that are hypothesized to function in the Ae. *aegypti* OR regulatory matrix. Each TF is expressed in a subset of *Ae. aegypti* antennal ORNs, and expression levels of each TF varies from neuron to neuron within this subset. Searches for consensus binding site sequences for the TFs uncovered multiple binding sites residing in the 5′ flanking sequences and first introns of multiple OR genes. The transcription factor Single-minded (Sim) has binding sites in ~50% of the OR genes, suggesting that it may function as a major regulator of OR expression. To functionally test this hypothesis, we used chitosan/siRNA nanoparticles to target sim during olfactory development. These experiments demonstrated that Sim regulates expression of a subset of OR genes and functions in the *Ae. aegypti* OR regulatory matrix. These findings correlated with an odorant tracking behavioral defect. Interestingly, *sim* knockdown animals also displayed severe antennal lobe defects, including improper ORN targeting and projection neuron defects coincident with a collapse in the structure and shape of the antennal lobe and individual glomeruli. The results of this investigation demonstrate that Sim functions in the coordinate regulation of OR expression and ORN targeting, two processes that dictate what odors will be detected by a neuron and which behaviors are elicited in response to these odors.

946

MALE-FEMALE MOLECULAR INTERACTIONS SHAPING THE REPRODUCTIVE SUCCESS OF *ANOPHELES GAMBIAE* MOSQUITOES

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The high reproductive rate of *Anopheles gambiae* mosquitoes is one of the principal components of their competence as malaria vectors, and is a target for novel vector control strategies aimed at reducing malaria transmission. Important biological processes underlying fecundity and fertility rates of *An. gambiae* mosquitoes are started by the interactions between male seminal fluid transferred during mating and female molecular pathways modulated by copulation. These yet unknown interactions trigger major changes in the physiology and behavior of females, including a reduced receptivity to further copulation, an increased egg production and the induction of egg laying. Here we identify a cascade of molecular events triggered by mating that increases egg development and facilitates egg laying in *An. gambiae* females. Using a combination of high-throughput studies followed by in depth functional analyses, we have identified a female Mating-Induced Stimulator of Oogenesis (MISO) that regulates the increase in egg development observed after mating and blood feeding. We also determine that MISO is regulated by and interacts with male factors transferred during copulation, and that this interaction is required for the correct accumulation of lipids in the developing oocytes. Moreover, by preventing the coagulation of seminal secretions in the male and their transfer to the female, we present evidence of the mechanisms inducing oviposition in these mosquitoes. These findings increase our knowledge of biological processes important for mosquito reproduction, and reveal possible targets for the design of novel tools for the control of natural vector populations.

947

THE VIRULENCE OF CHAGAS DISEASE AGENT *TRYPANOSOMA CRUZI* IN ITS INSECT VECTOR

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Avoiding the over-exploitation of the resources in a food patch while still obtaining all the resources needed to live and reproduce is a common challenge among all creatures. For many parasites that live inside another organism this is especially tricky because they do not have the option of moving to a new patch should they be too virulent and overexploit their current one. The classic idea is that the avoidance of host death should favor intermediate levels of parasite virulence, however it is known that this is not the case: parasites actually exhibit a wide range of virulence. In our work we investigate parasite co-infection and strain differences as drivers of virulence variation in Chagas disease vector *Rhodnius prolixus* and two of its parasites *Trypanosoma cruzi* and *T. rangeli*. We found that *T. cruzi-T. rangeli* co-infection significantly reduces the survival of *R. prolixus* up to 30 days post-infection, indicating that this co-infection could be removing *T. cruzi*-infected vectors from the Chagas disease transmission cycle before it is transmitted. We have also found that *T. cruzi* virulence in *R. prolixus* is highly variable, with *R. prolixus* death ranging from 2-80% depending on *T. cruzi* strain (all DTU I). Our aim is to provide insights into the ecology of the Chagas disease transmission cycle and ultimately contribute to strengthen Chagas disease prevention efforts.

948

VERTICAL TRANSMISSION BIOLOGY AND LOCALIZATION OF *NEORICKETTSIA RISTICII* IN LIFE CYCLE STAGES OF THE DIGENEAN *PLAGIORCHIS ELEGANS*

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Neorickettsia are obligate intracellular bacterial endosymbionts of digeneans. These endosymbionts pass through all stages of digenean life cycles via vertical transmission. They may also be passed from digeneans to vertebrates via horizontal transmission and cause diseases such as Sennetsu fever in humans, Potomac horse fever and salmon dog poisoning. Despite the role of these bacteria as pathogens in humans, domestic animals and wildlife, there is much to learn about their diversity, host associations and fundamental biology. Practically nothing is known about the quantitative aspects of their transmission or their distribution in digenean host tissues. We have screened digenean cercariae from snails collected in eastern North Dakota for the presence of *Neorickettsia* using real time PCR. We found two digenean species infected with *Neorickettsia*: *Plagiorchis elegans* with *N. risticii* and *Diplostomum sp.* with *Neorickettsia sp.* We have established the first laboratory experimental model of *Neorickettsia* using cercariae of *P. elegans* from the nature as a starting point. We were able to sustain *Neorickettsia* throughout several complete life cycles of *P. elegans*. The system included aquatic snails, *Lymnaea stagnalis,* as the first intermediate host, mosquitoes, *Culex pipiens,* as the second intermediate host and hamsters, *Mesocricetus auratus,* as the definitive host. The experimental model allowed us to quantify the efficiency of vertical transmission through the digenean life cycle (using real time PCR) and study the details of *Neorickettsia* localization in all developmental stages of digeneans (using immunofluorescent microscopy). Vertical transmission efficiency of *N. risticii* was invariably lower than 100% in cercariae and eggs, but reached 100% in sporocysts. The localization of *N. risticii* in organs and tissues of digeneans provides explanations for the differences in the infection rates among different stages.

949

THE DYNAMIC NATURE OF THE *SCHISTOSOMA MANSONI* APICAL TEGUMENT MEMBRANE IS DEPENDENT ON CYTOPLASMIC MOTOR PROTEINS

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The tegument of *Schistosoma mansoni* serves a variety of critical biological roles for the parasite, including nutrition, osmoregulation, immune evasion and immuno-modulation. Dynein light chains (DLCs) and tetraspanins (TSPs) are prominent proteins in the tegument and appear to be intimately linked with other molecules present in the apical, host-interactive, membrane of the parasites. In other eukaryotic cells, cytoplasmic DLCs are a subunit of motor complexes involved in diverse cellular translocation events as protein trafficking, mitosis and ciliary beating. DLCs of the schistosome tegument likely have similar roles and are likely to be important in the development and renewal of the tegument membrane, thereby contributing to parasite survival in its host. TSPs are membrane-spanning proteins that act as scaffolds for the formation of membrane-associated protein complexes comprising a wide variety of proteins. These TSP-enriched microdomains are known to play a wide range of roles within human cells, including maintenance of cell morphology and cell signalling. As an anti-schistosomal vaccine SmTSP-2 has shown high protection, however its biological role in the tegument remains unknown. To explore protein interactions within the tegument we have used multiple methods, including Blue Native polyacrylamide gel electrophoresis and protein crosslinkers, coupled with in-line liquid chromatography-tandem mass spectrometry. To functionally characterise selected tegumental proteins, RNA interference has been be used to knock down the gene of interest, and phenotypic changes are visualised by electron microscopy. This study provides insights into the molecular complexes associated with the host-interactive surface of schistosomes and will highlight which molecules are associated with the surface membranes and how these molecules functionally contribute to the maintenance of the parasite host-parasite interface. Tegument development and maintenance are critical biological functions for the survival of the parasite within the mammalian host and this research may lead to the identification of novel targets for vaccination or drug therapy for schistosomiasis.

950

DEVELOPMENT OF SEROLOGICAL TECHNIQUES FOR THE DIAGNOSIS OF ZOONOTIC *SCHISTOSOMA JAPONICUM* INFECTION THROUGH THE USE OF RECOMBINANT PROTEINS

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Schistosomiasis continues to be a public health problem in endemic countries. In the Philippines, schistosomiasis is endemic in 28 provinces caused by the zoonotic parasite *Schistosoma japonicum.* The role of animal reservoirs on the parasite's life cycle was not given much importance which might be the main hindrance in the possible elimination of the disease. Disease surveillance therefore should include both human and animals that might harbor and continually transmit the infection. In this study, we aim to develop a more sensitive and specific serological diagnosis for schistosomiasis both for human and animal host using recombinant proteins. Thioredoxin peroxidase-1 (SjTPx-1) and four tandem repeat proteins (Sj1TR, Sj2TR, Sj4TR, Sj7TR) using enzyme-linked immunosorbent assay (ELISA) were tested to determine their serological importance in human, water buffalo and dog diagnosis. Based on the results, SjTPx-1 might be used as a 'universal' diagnostic antigen that can detect human

and animal schistosome infection, whereas Sj7TR for both human and dog diagnosis and Sj1TR for water buffalo diagnosis. These results might encourage the development of a more accurate diagnostic test both for humans and animal schistosome infection based on the recombinant antigens and will therefore improve the disease surveillance leading to the possible elimination of this neglected parasitic disease in endemic countries like the Philippines.

951

EXPLORING SCHISTOSOMIASIS DIAGNOSIS USING MAGNETIC SEPARATION TECHNOLOGIES

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There is a great need for reliable and sensitive diagnostic tests for schistosomiasis that are affordable and able to be applied in field and laboratory settings. We have shown that magnetic beads bind strongly to schistosome eggs and can be used to manipulate and purify the eggs. The interaction between the beads and eggs is intriguing and of potential value in diagnosis. Adult schistosomes live in the vasculature of their human host, where they feed extensively on blood. Females are adapted to process large quantities of blood cells, which are digested using extracellular and epicellular digestive strategies. In addition to their requirements for catabolic products of haemoglobin, schistosomes also have nutritional dependence on many other metabolites and trace elements, notably iron (Fe). A number of pathways for Fe and haem uptake, transmembrane transport and storage have been identified for schistosomes. Schistosomes store abundant quantities of iron in vitelline glands, a follicular network of cells that synthesise precursors for eggshell formation. We have shown that this Fe store is most likely used during eggshell formation, becoming incorporated into the matrix and as iron-phosphate particles within pores of the shell. In this presentation, we will outline our studies on Fe and haem metabolism in schistosomes, focusing specifically on fate of Fe in embryogenesis. We will then discuss possible mechanisms for the strong interactions between magnetic beads and schistosome eggs and how this may be exploited for future diagnostic developments.

952

NEW GOLD STANDARD OF SCHISTOSOME DIAGNOSIS WITHOUT USING STOOL

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Schistosomiasis is a worldwide communicable disease caused by several species of genus Schistosome can be easily transmitted to human host. Clinical diagnosis of two major human schistosomes, *Schistosoma mansoni* and *S. haematobium* lacks sensitivity and is cumbersome to conduct. So control strategies based on targeted mass drug administration (MDA) to succeed it is essential to have a simple, easy to operate, sensitive and accurate test. The standard diagnostic tests, including stool examination by Kato-Katz (KK), detection of egg (dipstick) and blood (haematuria) in urine lack sensitivity, especially in low endemic settings. We compared diagnostic efficacy of KK, haematuria and polymerase chain reaction (PCR) based on species specific DNA to detect *S. mansoni* and *S. haematobium* infection from 86 filtered urine samples collected in Ghana from low endemic area. Because, these low level infections will maintain the reservoir of infection and many infections will still persist following mass chemotherapy. It is important to detect and treat such infections. Our approach with PCR to amplify DNA from urine showed promising signs with much higher sensitivity (ranges from 99% - 100%) and specificity (100%) compared to KK and haematuria (sensitivity: 76% and 30%) for both schistosome species detection. High positive and negative predictive values (90% - 100%) were also indicative of robustness of PCR. The same pattern was observed when stratified for age group and sex specific analysis. In addition PCR detected 11 such individuals infected for both parasites who were considered not infected by either parasite. We have demonstrated that parasite specific DNA can be detected in urine when some specimens are apparently negative, as presence of DNA in the urine indicates a viable infection is still present. Our approach of disclosure of schistosome infection from filtered urine samples by PCR can be an effective means to detect low intensity infection and would enhance the effectiveness of surveillance and MDA control programs of schistosomiasis.

953

SCHISTOSOME ABC MULTIDRUG TRANSPORTERS: ROLES IN PARASITE PHYSIOLOGY, DRUG SUSCEPTIBILITY AND IMMUNOMODULATORY SIGNALING

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Members of the ATP-binding cassette (ABC) superfamily of proteins are efflux transporters that remove toxins and xenobiotics from cells. They likely play key physiological roles in excretion of wastes and metabolites, and they also transport a variety of signaling molecules, including those that have immunomodulatory activity. ABC transporters were originally characterized based on their role in mammalian multidrug resistance (MDR), and changes in structure or expression of these proteins are also associated with drug resistance in parasites, including helminths. Based on these properties, we postulate that these proteins can be considered attractive candidate targets for novel antischistosomal agents. We have previously shown that expression of two *Schistosoma mansoni* ABC transporters, P-glycoprotein (Pgp; SMDR2) and multidrug resistance associated protein (MRP1; SmMRP1) is altered in worms exposed to praziquantel (PZQ), the current drug of choice against schistosomiasis. Higher basal expression of these proteins is found in schistosomes with reduced PZQ susceptibility, including isolates exhibiting resistance to PZQ. PZQ inhibits SMDR2, and is also a likely substrate, and knockdown and pharmacological experiments indicate that SMDR2 and SmMRP1 play a role in schistosome egg production. In current experiments, we are using genetic and pharmacological approaches to assess whether disruption of parasite multidrug transporter expression or function enhances PZQ activity against adult and juvenile worms. We are also using similar approaches to assess the role these transporters presentation of parasite molecules that influence the host's immune response.

954

GENETIC BASIS OF DRUG RESISTANCE AND SPECIES-SPECIFIC DRUG ACTION IN SCHISTOSOME PARASITES

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Oxamniquine kills *Schistosoma mansoni* but not *S. haematobium* or *S. japonicum*. High level resistance to oxamniquine evolved in the human blood fluke, *S. mansoni,* in Brazil in the 1970s and has been selected in laboratory populations. We exploited the genome sequence and genetic

map to identify the mutations underlying this trait, to determine the mode of drug action and the basis for species specific drug action. We staged a cross between parental parasites differing ~500-fold in drug response, determined drug sensitivity in clonally-derived F2s, and identified a single QTL (LOD=31) on chromosome 6. The causative gene, which encodes a sulfotransferase (SmSULT), was identified using RNAi knockdown and biochemical complementation assays, and we demonstrate independent origins of loss-of-function mutations in field-derived and laboratory-selected resistant parasites. By identifying the gene and specific mutations underlying drug resistance, we were able to confirm the mechanism of action. SmSULT sulfonates oxamniquine by catalyzing the transfer of a sulfuryl group (SO_3) from the active sulfate donor such as 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to oxamniquine to activate the drug, which binds to schistosome DNA, interfering with DNA synthesis and transcription, and killing the adult worms. In the laboratory-selected resistant parasite (HR) an amino acid deletion close to the active site interferes with drug binding, while in the field derived resistant isolate (MAP) a C→R mutation disrupts enzyme tertiary structure. These results demonstrate the utility of linkage mapping in a major human helminth parasite. Ongoing crystallography studies of protein-drug interactions demonstrate the structural relationship between the sulfate donor (PAPS), the sulfotransferase (SmSULT) and the drug (Oxamniquine), while phylogenetic studies revealed close homologues of the incriminated *S. mansoni* gene in *S. haematobium* and *S. japonicum*. These studies pave the way for rational design of second generation oxamniquine derivatives that can kill all human-infective schistosome species.

955

IMMUNE RESPONSES TO CHOLERA: LESSONS LEARNED FROM CHILDREN

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Cholera is an acute dehydrating diarrheal disease caused by *Vibrio cholerae* O1 or O139 infection. In endemic areas, young children bear a high burden of disease; however, currently available oral cholera vaccines (OCV) give a lower efficacy and shorter duration of protection in this group than in older children and adults. The immunological reasons responsible for this are unclear. We have shown that in natural infections, young children achieve comparable antigen-specific antibody, gut-homing antibody secreting cell, and memory B cell (MBC) responses as adults. Conversely, children given OCV (Dukoral) are unable to develop detectable MBC responses against *V. cholerae* lipopolysaccharide (LPS), though significant increases in cholera toxin subunit B (CtxB)-specific MBC are seen. Notably, when compared to age-matched patients, child vaccinees have significantly lower, or absent, antibody and MBC responses to LPS and the O-specific polysaccharide, its major antigenic component. Furthermore, in these child vaccinees, increased CtxB-specific antibody avidity persists beyond one year, but avidity against LPS falls to baseline levels by one month after completion of vaccination. We also show that older child vaccinees mount significant CtxB-induced effector memory T cell (Tem) responses accompanied by prominent Th1 and Th2 cytokine secretion. Such Tem responses correlate with subsequent MBC levels up to 30 days, as well as increased antibody avidity up to one year. Younger child vaccinees, on the other hand, exhibit a lack of Tem response, and instead mount a prominent Treg response. Our findings suggest that young children given the OCV mount a different T cell response to the T-dependent antigen CtxB than older children, and most importantly, are unable to mount a durable memory immune response to LPS, the antigen believed to be most important in protection. These findings may explain the lower level of protection afforded to young children by vaccination.

956

ENVIRONMENTAL SURVEILLANCE FOR TOXIGENIC *VIBRIO CHOLERAE* IN SURFACE WATERS OF HAITI

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The cholera epidemic that began in Haiti in 2010 has sickened more than 500,000 people. As outbreaks of cholera were not previously reported in Haiti, there is no information regarding the environmental presence of toxigenic *Vibrio cholerae* in Haitian surface waters. During each of 4 field visits (October 2011, March and August 2012, and January 2013), 19 river, canal, lake, and marine water sites were surveyed for the presence of toxigenic *V. cholerae*. Water samples were collected by 1-L grab sampling, 100-L dead-end ultrafiltration, and 100-L plankton net sampling. Samples were enriched overnight in alkaline peptone water (APW) and TCBS agar was used to isolate *V. cholerae* colonies. Real-time PCR assays targeting the *omp*W, *ctx*A, and *tcp*A genes were used to identify potential toxigenic *V. cholerae* isolates. APW enrichments were also screened directly for the presence of cholera toxin gene sequences (*ctx*A). Toxigenic *V. cholerae* was isolated from a river in the Artibonite Department in October 2011 and again from a different river in the same department in January 2013. Whole genome sequencing revealed that these isolates were a match to the outbreak strain. Cholera toxin (*ctx*A) was detected at 11 sites in October 2011 and 5 sites each in August 2012 and January 2013. Cholera toxin was not detected from any site in March 2012. Results of this survey demonstrate that toxigenic *V. cholerae* is present in surface waters in Haiti more than two years after the onset of the epidemic.

957

CHOLERA EPIDEMIC ASSOCIATED WITH UNSAFE DRINKING WATER AND STREET-VENDED WATER - EASTERN FREETOWN, SIERRA LEONE, 2012

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Vibrio cholerae causes an estimated 3 million illnesses and 100,000 deaths annually. During 2012, Sierra Leone experienced a severe cholera epidemic with 22,252 reported cases and 292 deaths. In August 2012, CDC assisted the Ministry of Health and Sanitation in an outbreak investigation. The investigation team conducted a matched case-control study to assess risk factors for cholera. Cases were defined as acute watery diarrhea requiring IV hydration in persons ≥5 years old, presenting to a health facility from September 10-21. Controls were matched by age and neighborhood. Stool specimens from case-patients were analyzed by culture and polymerase chain reaction (PCR) for *V. cholerae*; isolates were subtyped by pulsed-field gel electrophoresis (PFGE). Conditional multivariate logistic regression was performed to investigate cholera risk factors. We enrolled 49 cases and 98 matched controls. Virtually all cases (96%) and controls (96%) obtained drinking water from improved water sources, such as boreholes and public taps. Consuming unsafe water (matched odds ratio [mOR]: 3.4; 95% confidence interval [CI]: 1.1, 11.0), street-vended water (mOR: 9.4; 95% CI: 2.0, 43.7) and crab (mOR: 3.3; 95% CI: 1.03, 10.6) were significant risk factors for cholera infection. Of 31 stool specimens from cases, 13 (42%) showed PCR evidence of toxigenic *V. cholerae*. Three specimens yielded isolates of *V. cholerae* O1, El Tor; all were resistant to co-trimoxazole and susceptible to doxycycline, ciprofloxacin, and tetracycline. PFGE analysis identified a pattern previously observed in seven countries. Testing of stored drinking water demonstrated evidence of chlorination in 28% of case and 38% of control households. Despite

near universal access to improved water sources, consuming unsafe water and street-vended water were risk factors for cholera infection. We recommended that prevention efforts focus on enhancing the microbiologic quality of improved water sources, promoting household drinking water chlorination, and improving street vendor water handling practices.

958

OBSERVATIONS FROM THE MASS VACCINATION PROGRAM WITH THE ORAL CHOLERA VACCINE, SHANCHOL, IN A HIGH RISK URBAN SETTING IN DHAKA, BANGLADESH 2011-2013

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A large feasibility study on an oral cholera vaccine, Shanchol was conducted in a high risk urban population in Mirpur in Dhaka, Bangladesh. The aim was to determine to the feasibility of delivery and vaccination strategies utilizing the existing national immunization system. Bangladesh faces biannual peaks of cholera each year with 450,000 severe cases and at least a million infections. The need to utilize an affordable cholera vaccine as a public health intervention in a cholera prone country appeared extremely important in the national and regional context. The design of the feasibility study included vaccination together with a behavior change communication strategy in urban Mirpur, in six wards with a high cholera hospitalization rate. Following a geographic information system (GIS) approach, a census was carried out and the study was conducted using a cluster randomized design. Consent was obtained from eligible participants and bar coded identification cards were provided to individuals for census updates and to identify them during passive surveillance for cholera. Three arms of the program included a vaccine (n=80,000), a vaccine plus behavior change communication (n=80,000) as well as a non-intervention control arm (n=80,000). The vaccination program with Shanchol was conducted from the 17th of February to the 16th of April, 2011. Of the 171,110 target population (excluding those aged <1yr and pregnant woman), vaccination was carried out in about 141,882 individuals. About 87% of coverage was obtained for two doses of the vaccine. Thus, over 265,577 doses of Shanchol was delivered in less than two months using fixed site vaccination as well as mop up activities following the EPI procedure. Passive surveillance for detecting culture confirmed cholera cases were based on visits by participants to health facilities. The study has completed two years of surveillance in April 2013. Results of the effectiveness of the interventions and lessons learnt from the feasibility study so far will be presented.

959

COMPARATIVE PROTEOMIC ANALYSIS REVEALS ACTIVATION OF INNATE IMMUNE SIGNALING PATHWAYS AND THE INFLAMMASOME IN BANGLADESHI CHOLERA PATIENTS

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Cholera, caused by the bacterium *Vibrio cholerae*, is responsible for 3-5 million cases of diarrheal disease per year. Genome-wide studies of cholera patients in Bangladesh show that host genetic characteristics play an important role in susceptibility. These "omics" studies elucidate the poorly understood interaction between *V. cholerae* and the human host and provide insight for vaccine development. We collected duodenal biopsies from cholera patients in Bangladesh, 2 days post infection and again 30 days later when symptoms were absent. We analyzed tissue samples using label-free mass spectrometry (MS/MS) and MaxQuant, a protein quantification program, and compiled a list of 121 proteins that were differentially abundant ($p < 0.05$). Of those proteins, 34 were immunologically relevant including innate defense proteins previously identified in a study of acute cholera. We then used pathway analysis software to identify molecular interactions among proteins in our dataset and between other proteins in a reference database. Several proteins in our dataset were connected to innate immune pathways involved in TLR4-IFN signal transduction, NF-kB activation, and regulation of the NALP3 inflammasome. We confirmed differential abundance of two candidate proteins, WARS and S100A8, using immunohistochemistry. Involvement of WARS in *V. cholerae* infection and innate immunity has not previously been examined, and we speculate that WARS may activate certain pathways in a novel, cholera-specific manner. Recently, we have been developing an *in vitro* cell culture model of *V. cholerae* infection and we intend to use RNA interference in the context of this model to perform knockdown studies of several candidate proteins, including WARS and S100A8. Using this combined *in vivo* and *in vitro* approach, we can elucidate the role of these proteins in cholera pathogenesis and contribute to the understanding of human-*Vibrio* interaction at the gut mucosal surface.

960

THE RELATIONSHIP BETWEEN THE ANTIBIOTIC RESISTANCE AND VIRULENCE OF *ESCHERICHIA COLI*: A COMMUNITY-BASED STUDY IN RURAL ECUADOR

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Antimicrobial resistance is one of the most pressing issues in global health. Resistance can occur in any bacteria, but resistance in virulent bacteria (i.e. pathogens) is more threatening because of concerns about the consequences of an untreatable infection. Resistance may be linked to virulence as the result of differential antibiotic selection pressure and physical linkages between resistance genes and virulence genes. However, such relationships have rarely been evaluated with a community-based epidemiological study. In this case-control study of diarrheal diseases in northwestern Ecuador during 2009-2010, we systematically identified both pathogenic (n=86) and commensal (n=761) *Escherichia coli* isolates from 25 rural villages. Using antibiogram data for 12 antibiotics, *E. coli* genotypes, and resistance and virulence gene data, we assessed whether antibiotic resistance is linked with virulence in E .*coli* and explored factors that might contribute to such linkage. We observed that resistance to both individual and multiple antibiotics was higher in *E. coli* isolated from cases than from controls, and even higher in pathogenic versus commensal *E. coli*. Using a generalized estimating equation we found that pathogen status was more strongly related to antibiotic resistance than case status or antibiotic use for all antibiotics except quinolones. There was only a weak positive correlation between three out of four resistance genes and the virulence genes we tested. But pathogens and commensal *E. coli* differed significantly with regard to phylogroup distribution. These data suggest that the virulence and antibiotic resistance in *E. coli* are linked phenotypes, and the genetic background likely contributes to this linkage. Antimicrobial stewardship in the community setting is needed to address the concern that antimicrobial use may contribute to the persistence and spread of more virulent bacteria.

961

MAPPING THE EPIDEMIOLOGY OF YAWS IN THE SOLOMON ISLANDS

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Yaws is a re-emerging endemic treponemal infection, with the Pacific Islands a major focus of infection. The WHO is aiming to eradicate yaws by 2020 with a strategy that is heavily reliant on single-dose azithromycin treatment but there are scarce detailed epidemiological data to guide these efforts. Surveys of the Solomon Islands are ongoing to map the burden of disease. Incidence of reported clinical yaws in 2011 was 3,300 cases per 100,000 increasing to 5,382 cases per 100,000 in children aged ≤14 although cases are not routinely confirmed with serology. Data currently available for 2012 suggest a similar incidence in that year. There is considerable geographic variation within the country with the incidence varying between provinces from 1,353 to 8,418 cases per 100,000. These figures are likely to represent an underestimate of the true burden of disease. We have carried out population-based prevalence surveys in each of two provinces (Western and Choiseul) providing detailed data on both active and latent yaws infection. Over 1,000 children per province have been enrolled. A standardised questionnaire and examination were used to collect clinical data on both primary and secondary yaws. Whole blood was collected for serological testing (TPPA and RPR) on each subject, with a T. pertenue-specific PCR undertaken on individuals with suspected yaws ulcers and, a PCR to detect mutations associated with azithromycin resistance in *Treponema pallidum*. Independent review of skin-lesion photographs allows detection of additional skin disease phenotypes. We will present data on the prevalence of primary, secondary and latent yaws infection and the frequency of mutations conferring resistance to azithromycin. This work contributes significantly to our understanding of the epidemiology of both yaws and other skin diseases in the Solomon Islands and provides important data to inform yaws eradication efforts.

962

EVALUATION OF A MULTIVALENT VACCINE AGAINST LYMPHATIC FILARIASIS IN RHESUS MACAQUE MODEL

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Lymphatic filariasis affects 120 million people around the world and another 1.2 billion people are at risk. Chemotherapy with mass drug administration is substantially reducing the incidence of the infection. Nevertheless, an effective vaccine is needed to prevent the infection and eliminate the disease. Previously we reported that a multivalent fusion protein vaccine (rBmHAT) composed of small heat shock proteins 12.6 (HSP12.6), abundant larval transcript-2 (ALT-2) and large extracellular domain of tetrapsanin (TSP LEL) could confer >95% protection against *Brugia malayi* L3 challenge in mouse model. In this study we evaluated the immunogenicity and efficacy of the rBmHAT fusion protein vaccine in a Rhesus macaque model. A total of 10 rhesus monkeys were divided into two groups with five animals in each. Test animals received three intramuscular injections of 200µg of rBmHAT plus 200 µg of alum (IDRI) on days 0, 28 and 56. Control animals received only alum. Animals were bled after each dose and analyzed for vaccine induced antibody titers using an indirect ELISA. Our results show that all vaccinated monkeys developed significant titers of antigen-specific IgG antibodies (1:40,000) compared to controls. Antibody isotype analysis confirmed that the rBmHAT vaccination induced IgG1 production in response to all three antigens. Additionally, peripheral blood mononuclear cells from immunized animals proliferated significantly (S.I.-1.262±0.0738) in response to the vaccine antigen. Moreover, ELISPOT analysis confirmed that the antigen specific cells predominantly secreted IFN-γ. Finally, an *in vitro* antibody dependent cellular cytotoxicity (ADCC) assay showed that the antibodies in the sera of rBmHAT immunized animals participated in the killing of *Brugia malayi* L3 conferring 40% ±10.04 protection compared to controls. Taken together, this data is the first to demonstrate vaccine-induced protection against *B. malayi* in non human primates.

963

TARGETING IMMUNOSUPPRESSION AS A VACCINE STRATEGY AGAINST CHRONIC HELMINTH INFECTION

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The ability of helminths to ensure their survival and transmission rests upon their successful suppression of host immunity months- to decades-long. We predicted that by depleting immunomodulatory proteins which these parasites secrete, we could restore protective immune function to the host. Using *Litomosoides sigmodontis*, a filarial nematode that can complete its life cycle in laboratory mice, we reduced burdens of sexually mature adult parasites by 60-80% and their offspring by 90% using DNA vaccines expressing two genetically modified immunomodulatory proteins: abundant larval transcript LsALT-1 and cystatin LsCPI-2 of which we inactivated the immunosuppressive functions and targeted them specifically to dendritic cells (DC) using a sequence that encodes an anti-DEC205 single chain antibody. Unlike attenuated vaccines, which act immediately upon infection but not thereafter, the anti-immunomodulatory vaccines induced a gradual killing of *L. sigmodontis*. We have now identified several immune mechanisms *in vivo* and *in vitro* that these vaccines trigger, including IL4-independant increased production of IL12 and IL6 by DC, and increased proliferation of CD4+ T cells prior to challenge. During the chronic phase of infection, the same vaccine formulations, which also include host MIP1α- and IL4-expressing plasmids, induced stronger type-2 cellular and humoral immunity that correlated with worm killing. In conclusion, our results support our prediction that anti-immunodulatory vaccination would restore host immune function, and point to the importance of DC activation upon first encounter with helminths in building up protective immunity.

964

CHRONIC HELMINTH INFECTION PROTECTS MICE FROM TYPE III HYPERSENSITIVITY

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The mechanisms by which helminth infections protect against inflammatory diseases are not fully understood. In this study, we evaluated the effects chronic helminth infections have on type III hypersensitivity. Type III hypersensitivity, such as an Arthus reaction or serum sickness, is driven by antigen/antibody immune complex deposition and plays an important role in the pathogenesis of numerous diseases, including systemic lupus erythematosus. Mice were sensitized to ovalbumin (OVA) and then infected with *Litomosoides sigmodontis* for 10 weeks. Chronically infected animals exhibited significantly reduced ear swelling 12-24 hours after intradermal ear challenge with OVA ($p < 0.001$). Experiments using mice deficient in or depleted of CD4+ cells, mast cells, basophils, antibodies, or IgE demonstrated that this late phase inflammation was due to immune complex-mediated

type III hypersensitivity. Although total IgG and IgE levels were higher in sensitized + infected mice than sensitized mice, OVA-specific IgG1 ($p < 0.01$), IgG2a ($p < 0.05$), and IgE ($p < 0.001$) were all significantly reduced in mice that were chronically infected. After intradermal OVA challenge, immune complexes were detected by fluorescence microscopy in the tissues of both sensitized and sensitized + infected mice, with those in sensitized + infected mice larger and more focal than those in sensitized mice. Histological analysis of sensitized + infected mice showed reductions in edema, necrosis, and neutrophil and eosinophil numbers compared to sensitized animals. Although chronic helminth infection reduced circulating levels of C3 ($p < 0.01$), sensitized + infected mice exhibited the same kinetics of C3 decrease after OVA challenge as sensitized mice. In conclusion, these data demonstrate that a 10 week *L. sigmodontis* infection protects against type III hypersensitivity responses. Given the importance of immune complex reactions in the pathogenesis of numerous inflammatory diseases, these findings suggest a new mechanism by which helminth infections protect against inflammatory diseases. Future studies aim to further elucidate the immunological pathways mediating protection against type III hypersensitivity, and to evaluate whether worm-derived therapies can be useful in protection against diseases in which immune complex deposition is involved.

965

CHRONIC FILARIAL INFECTION IMPROVES *ESCHERICHIA COLI* INDUCED SEPSIS IN A TLR2 DEPENDENT MANNER

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Helminths modulate the immune system of their hosts and induce a regulatory, anti-inflammatory milieu that enables long-term parasite survival within the host, but may also benefit the host. Thus, helminth infections have been shown to improve allergies and autoimmune diseases. In the current study we investigated whether chronic infection with the filarial nematode *Litomosoides sigmodontis* (L.s.) improves the outcome of an acute systemic inflammation caused by i.p. *Escherichia coli* injection in BALB/c mice. Chronic L.s. infection significantly improved *E. coli* induced hypothermia, bacterial clearance and sepsis survival compared to *E. coli*-only injected controls. The L.s. mediated protective effect correlated with significantly increased levels of anti-inflammatory TGFβ and reduced concentrations of pro-inflammatory cytokines after *E. coli* challenge. Depletion of peritoneal macrophages prevented the filaria mediated protection against *E. coli* challenge. Improved bacterial clearance in L.s. infected animals was not due to an increased phagocytic capacity of macrophages, but correlated with a reduced loss of peritoneal macrophages and an alternatively activated phenotype after *E. coli* challenge. However, the L.s. mediated protective effect was still given in IL-4 and IL-4R/IL-5 deficient animals, suggesting that alternatively activated macrophages are not required. Endosymbiotic *Wolbachia* bacteria, that are present in most human pathogenic filaria and L.s., signal via TLR2 and may induce cross-tolerance to TLR4 stimuli. Accordingly, *in vitro* experiments revealed that pretreatment of macrophages with crude L.s. antigen reduced LPS induced activation and lack of TLR2 signaling in L.s. infected mice prevented the protection against *E. coli* challenge. Our study suggests that chronic L.s. infection can have a beneficial effect on acute bacterial infections. Current experiments are ongoing to test whether this is due to exposure to *Wolbachia* bacteria in chronically L.s. infected mice that prevents *E. coli* induced excessive macrophage activation by a TLR cross-tolerance mechanism.

966

IDENTIFICATION OF HUMAN TH9 CELLS: ANTIGEN-SPECIFIC, IL-4- AND TGFβ-DEPENDENT EXPANSION OF A DISCRETE NON TH2 CD4+ T CELL SUBSET AND THEIR ROLE IN A CHRONIC FILARIAL INFECTION

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Th9 cells are a subset of $CD4^+$ T cells producing IL-9 and IL-10 and shown to be important in allergy, autoimmunity and antitumor responses. However, their role in human infectious diseases has not been explored in detail. Lymphatic filariasis can be associated with the development of serious pathology in the form of lymphedema, hydrocele and elephantiasis in a subset of infected patients. We postulated that this infection would provide an ideal milieu to examine the role of Th9 cells. We identified a population of IL-9 and IL-10 co-expressing $CD4^+$ T cells (lacking IL-4 expression) in normal individuals that respond to antigenic and mitogenic stimulation but are distinct from $IL\text{-}9^+$ Th2 cells. We also demonstrate that these Th9 cells exhibit antigen -specific expansion in filarial infected individuals. Comparison of Th9 responses reveals that individuals with lymphedema and elephantiasis associated with *Wuchereria bancrofti* infection exhibit significantly ($p<0.0001$) expanded frequencies of filarial antigen induced Th9 cells but not of $IL9^+$Th2 cells in comparison to filarial-infected individuals without associated disease (subclinical infection). Moreover, the per cell production of IL-9 ($p<0.0001$) is significantly higher in Th9 cells compared to $IL9^+$Th2 cells, indicating that the Th9 cells are the predominant $CD4^+$ T cell subset producing IL-9 in the context of human infection. The expansion of $IL\text{-}9^+$ $CD4^+$ T cells was also reflected in elevated antigen stimulated IL-9 ($p<0.0001$) cytokine levels in whole blood culture supernatants. Finally, the baseline and antigen driven frequencies of Th9 cells correlated positively with the severity of lymphedema (and presumed inflammation) in filarial diseased individuals. This antigen driven expansion of Th9 cells was dependent on IL-4 and TGFβ as blockade of these two cytokines resulted in a significantly decreased expansion of Th9 cells in short term cultures. We have therefore identified an important human $CD4^+$ T cell subpopulation co-expressing IL-9 and IL-10 but not IL-4, whose expansion is associated with disease in chronic lymphatic filariasis and could potentially play an important role in the pathogenesis of many other inflammatory disorders.

967

THE EXPANDED REGULATORY T CELL POPULATION IN CHRONIC FILARIAL INFECTION IS HIGHLY HETEROGENEOUS, ACTIVATED AND SUPPRESSES CYTOKINE PRODUCTION DENDRITIC CELLS

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Natural regulatory T cells (nTregs) are known to increase during chronic infection or at the site tumor, though the exact mechanisms that contribute to their accumulation and mode of action remain unclear. We used flow cytometry and microarray analyses to delineate the phenotype of nTregs and their role in modulating T cell and antigen presenting cell (APC) function in the setting of chronic infection. Using cells from 18 filaria-infected (Fil+) and 19 filaria-uninfected (Fil-) subjects, we found that the frequencies of nTreg expressing CTLA-4, GITR, LAG-3, and IL-10 were significantly higher in Fil+ compared with Fil- subjects. Microarray analysis revealed that, compared with those from Fil-, nTreg populations in Fil+ subjects were more heterogeneous and had higher expression of IL-10, CCL-4, IL-29 and CTLA-4, molecules that have been implicated in immune suppression. Moreover, the nTregs from Fil+ subjects had markedly upregulated activation-induced apoptotic genes with concomitant down regulation of cell survival genes. To determine if nTregs directly modulate APC function, we used an *in vitro* co-culture system with purified APCs and nTregs from Fil+ and Fil- subjects. In this system, in response to *Brugia malayi* antigen, APCs from Fil+ produced higher levels of IL-6 and TNF-α ($p = 0.01$ and $p = 0.04$, respectively). When APCs and nTregs were co-cultured together, in response to BMA stimulation APCs-nTregs co-culture from Fil+ only produced higher levels of IL-10 compared with APCs. Antibody blockade of nTregs surface makers (PD1, GITR, CTLA-4 and LAG-3) in nTregs-APCs co-cultures induced higher levels of IL-10 and TGF-β cells from Fil+ subjects only. Taken together, our results suggest that in filarial infection, the expanded nTreg populations are heterogeneous, short-lived, activated and express regulatory molecules that modulate cytokine production by APCs.

968

FAILURE OF HUMAN LANGERHANS CELLS TO INITIATE AN INFLAMMATORY RESPONSE FOLLOWING EXPOSURE TO FILARIAL INFECTIVE LARVAE (L3) SUGGESTS A MECHANISM FOR IMMUNE EVASION

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Epidermal Langerhans cells (LC) and keratinocytes (KC) most likely are the first line of defense following the interaction with the infective third larval stage (L3) of *Brugia malayi*. Previous studies from our laboratory indicate that 48 hours exposure to L3 failed to activate human LC but did, to a small degree, functionally suppress these cells. To assess whether longer exposure of epidermal LC to the parasite is required to induce an innate immune response, we used an *ex vivo* human skin blister model as well as *in vitro* generated LC. Our data indicate that longer L3 exposure (3-5 days) to human epidermal skin blisters (but not to the *in vitro* generated LC) downregulated the mRNA expression of thymic stromal lymphopoietin (TSLP) and significantly inhibited the production of TNFα ($p=0.02$) but not inflammasome-associated cytokines IL-18 and IL-1RA. The difference between the blisters and *in vitro* generated LC in TNF-α and TSLP downregulation by L3 may suggest for the role of KC in response to the parasite. Because toll like receptors (TLR) play an important role in pathogen recognition, we next determined the effect of L3 on LC TLR function. *In vitro* generated LC were exposed to live L3 (or other stimuli) and then assessed for responses to ligands for TLR-2, -3, -4, and -6) using Luminex™. Our data suggests that exposure to L3 did not alter the production of any of the cytokines measured including IL-10, IL-12p40, IL-1α, IL-1β, TNFα, and IL-6. Taken together, these data suggest that the muted response to L3 by LC even at longer exposure may be a mechanism by which the parasite escapes the consequences of the innate barrier associated response. Moreover, the presence of KC may be important in mediating the LC response to the parasite.

969

DIHYDROARTEMISININ-PIPERAQUINE VS. ARTEMETHER-LUMEFANTRINE FOR FIRST-LINE TREATMENT OF UNCOMPLICATED MALARIA IN AFRICAN CHILDREN: A COST-EFFECTIVENESS ANALYSIS

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Recent trials in Africa showed that dihydroartemisinin-piperaquine (DHAPQ), a newer fixed-dose artemisinin-based combination therapy (ACT), was as efficacious and safe as artemether-lumefantrine (AL) for treatment of young children with uncomplicated malaria across different endemicity settings. Longitudinal follow-up of patients also confirmed that DHAPQ had a longer post-treatment prophylactic effect than AL, reducing the risk of recrudescence and reinfection and hence conferring additional health benefits to patients. We estimated the threshold costs of DHAPQ per course treatment for which DHAPQ would be a cost-effective alternative to AL, given the post-treatment prophylactic effects of these drugs. Decision analysis was performed using a Markov model that simulated incidence of uncomplicated and severe malaria, survival, disability adjusted life years (DALYs) and costs in a hypothetical cohort of children receiving DHAPQ or AL for treatment of uncomplicated malaria. We calculated weekly hazard rates for recurrent malaria after treatment with DHAPQ and AL, using the published data on the proportion of patients whose treatment was failure free by day of follow up from a multi-center randomized control trial in Africa. At a mean cost of $0.36 per treatment course for both drugs, first-line treatment with DHAPQ was the dominant treatment strategy over AL with an improvement of 0.05 DALYs averted per child (95% CI 0.01--0.12) and a cost saving of $2.06 per child (95% CI 0.48--5.09). First-line treatment with DHAPQ remained the dominant treatment strategy (less costly and more effective) for any cost per course treatment below $1.27. Between $1.27 and $1.78, first-line treatment with DHAPQ was a "highly attractive" intervention compared to AL with an incremental cost-effectiveness ratio less than "$25 per DALY averted" under World Bank guidance. This study demonstrates the superiority of DHAPQ over AL for the treatment of uncomplicated *Plasmodium falciparum* malaria in young children from a clinical and economic perspective. Substituting DHAPQ with AL as first-line antimalarial treatment in highly endemic areas of Africa merits serious consideration by health policy makers.

970

EFFICACY AND ACCEPTABILITY OF ARTEMETHER-LUMEFANTRINE VERSUS DIHYDROARTEMISININ-PIPERAQUINE IN KENYAN CHILDREN WITH UNCOMPLICATED *FALCIPARUM* MALARIA

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Artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) have been introduced as first and second-line treatment, respectively, for uncomplicated *falciparum* malaria in Kenya. This open-label, randomized, comparative study in Western Kenya compared corrected Acceptable Clinical and Parasitological Responses (ACPR) in children aged 6 to 59 months, treated with AL-dispersible (AL_d) and DP-pediatric (DP_p). Adherence and acceptability of both drugs were also assessed. Children were hospitalized for 3-days to receive either AL_d (n=227) or DP_p (n=227) and followed up on Days 7, 14, 28 and 42. Adherence and acceptability were assessed by caregiver questionnaire, including general questions with respect to preferred pediatric formulations. No significant differences were observed for corrected ACPR rates on Days 14, 28 and 42 for AL_d (100%, 97.8%, and 96.8%, respectively) and DP_p (100%, 99.1%, and 98.7%, respectively; p>0.05). Similar results were seen for uncorrected ACPR rates. Overall incidence of adverse events was 65.5% (156/238) and 67.5% (156/231) in AL_d and DP_p arms. The adherence to treatment regimen was higher for children treated with AL_d (93.6%) compared to DP_p (85.6%). 82% of the caregivers considered AL_d 'simple' or 'very simple' to use compared with 67% in DP_p arm (p=0.007). The taste of AL_d was 'liked' or 'liked very much' by 72% of respondents, compared with 56% of respondents for DP_p (p=0.001). The majority in both groups took the drug with a meal (AL_d=94.4%; DP_p=89.4%), and preferred water to dissolve the tablets (AL_d=94.4%; DP_p=89.4%). In general, caregivers preferred the dispersible tablet formulation (drug given as tablet dissolved in a small volume of water/milk) as compared to a syrup formulation (AL_d=76.8% vs. 16.8%; DP_p=62.3% vs. 29.5%). Both, AL_d and DP_p are efficacious treatments for uncomplicated *falciparum* malaria in Kenyan children. Acceptability of AL_d regimen was assessed as being significantly better than DP_p in the caregiver survey.

971

TREATMENT EFFICACY OF ARTESUNATE-AMODIAQUINE TREATMENT REGIMENS FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA: COMPARISON OF FIXED VERSUS CO-BLISTER FORMULATIONS

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Artesunate-amodiaquine (AS-AQ) is the first line antimalarial treatment in 25 countries and it is available in non-fixed dose formulations (nFDC), either loose or co-blister. A fixed dose combination (FDC) of AS-AQ was introduced in 2007 to optimize the AS-AQ ratio and improve adherence. Current dosing for the FDC is according to 3 age ranges with 3 dosing strengths available. To assess the spectrum of total weight adjusted dosages (mg/kg) of AQ administered and to compare the effect of fixed and non-fixed AS-AQ combinations on treatment efficacy, individual patient data were shared with WWARN and collated using standardised methodology. Factors associated with Polymerase chain reaction (PCR)-confirmed recrudescences were assessed using a Cox's regression model with shared frailty on study sites. Data from 6,140 patients (26 efficacy studies, between 2002 and 2011) with uncomplicated *P. falciparum* malaria were included in the analysis [6,043 from Africa, 97 from Asia]. 22 studies (n=5,563) had a follow up of 28 days and 4 studies followed patients for 42 days or longer (n=577). 167 (2.7%) PCR-confirmed recrudescent parasitaemia were observed. Patients treated with the FDC received a higher median (IQR) mg/kg dose of AQ [33.8 mg/kg (27.5-40.5)] compared to those receiving nFDC [27.0 mg/kg (25.0-35.0)] (p<0.001). In a multivariate model, independent risk factors associated with recrudescence were: young age (1- <5 years old) (AHR=5.41 [95% CI: 1.58-18.47], p=0.007 compared to aged≥12 years) and log of the baseline parasitaemia (AHR=1.26 [95% CI: 1.10-1.43, p<0.001). The mg/kg dose of AQ was not a significant risk factor. After adjusting for confounding factors, the use of non-fixed dose formulations was associated with 2.8 fold increased risk of treatment failure [AHR: 2.85, 95% CI: 1.58-5.10, p<0.001]. The fixed dose formulation provides better efficacy than loose combinations, and this likely due to improved dosing. In addition, it is expected that FDC would improve adherence, effectiveness and eliminate the risk of using either drug as monotherapy, one of the drivers of resistance.

972

CARRIAGE OF DRUG RESISTANT *PLASMODIUM FALCIPARUM* IN MALARIA/HIV CO-INFECTED PATIENTS AND IMPROVEMENT OF CD4+ COUNTS AFTER ARTEMETHER-LUMEFANTRINE TREATMENT IN PORT HARCOURT, NIGERIA

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The occurrence of malaria in HIV infected persons is a major challenge to public health. HIV increases the risk of malaria infection and clinical malaria in adults in areas of stable transmission especially in those with advanced immunosuppression. HIV also increases frequency of drug intake and thus drug pressure. This may contribute to the emergence and spread of drug resistance. Higher parasite burdens as seen in HIV subjects may also increase the likelihood of carrying drug resistant parasites. Studies of molecular markers of antimalarial drug resistance have usually not included HIV subjects. The study was carried out in Port Harcourt, Nigeria, rich in the nation's oil resources and with high malaria transmission rates. Due to the oil and gas activities in the area, there is a large number of migrant workers with commercial sex workers following the camp resulting in high prevalence of HIV infection. Asymptomatic *Plasmodium falciparum* carriers were identified by microscopy among adult attendees at the HIV clinics and HIV-negative people from University of Port Harcourt Teaching Hospital community. Participants were treated with artemether-lumefantrine (AL)and followed up; blood samples were collected on D0, D3 and D28. Assaying for molecular markers was carried out and CD4+ T-cells counted. Paired peripheral blood CD4 cell counts at both day 0 and day 28 were available for 38 HIV volunteers. Over 28 days following, we observed a mean increase of 107.3 cells per µl (95% CI 53.8-160.8; P=0.0002). Using the PCR data as a more reliable test for parasite carriage, we found that 24 of these volunteers had confirmed parasites and 19 of these had cleared the parasites by D28 after treatment with AL. Genotype data at the pfmdr1, pfcrt and antifolate resistance marker *loci* pfdhfr and pfdhps will be presented.

973

PRIMAQUINE FAILURE IN *PLASMODIUM VIVAX* THERAPY IS CAUSED BY A DEFICIENCY IN CYTOCHROME P450 2D6

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Primaquine (PQ) is the only FDA-approved medication to treat the hypnozoites of *Plasmodium vivax*, but relapses due to drug failure occur. Human cytochrome P450 isoenzyme 2D6 (CYP2D6) metabolizes PQ and may play a critical role in production of active PQ metabolites in the liver. We sought to identify an association between CYP2D6 activity and PQ drug failure. 33 subjects were challenged with *P. vivax* sporozoites by the bites of infected mosquitoes. Beginning on the day of *parasitemia*, all subjects were treated for with a combination of chloroquine (1500 mg base over 48 hours) and PQ (30 mg base by mouth daily for 14 days) under directly observed therapy. Subjects were monitored for malaria relapse for >12 months. Upon relapse, chloroquine dosing was repeated; however, PQ dosing was weight-based to acheive a total dose of 6 mg/kg. CYP2D6 phenotypes were ascertained in 25 subjects. The pharmacokinetics of PQ metabolism was determined using plasma from these subjects. All subjects became parasitemic by day 13 post challenge and rapidly cleared *parasitemia* upon initiation of therapy. Two subjects (6%) experienced malaria relapses. CYP2D6 phenotyping revealed 21 (84%) extensive metabolizers (EM), 3 (12%) intermediate metabolizers (IM), and 1 (4%) poor metabolizer (PM). There were 0 relapses in the EM group, 2 relapses in one IM subject, and 3 relapses in the PM subject. Rapid clearance of blood stage parasites was documented by PCR following the initiation of re-treatment for relapses. There was a significant association between relapse and CYP2D6 phenotypes associated with low isoenzyme activity. Pharmacokinetic analysis demonstrated markedly higher plasma concentrations of parent PQ in relapse subjects consistent with decreased metabolism by CYP2D6. CYP2D6 activity appears to be critical in metabolizing PQ to its active metabolite. In this small population of subjects, deficiency of CYP2D6 isoenzyme activity was associated with failure of PQ to prevent relapse of *P. vivax*. Larger study populations are necessary to further elucidate the relationship between CYP2D6 activity, geographic regional dosing requirements, and clinical failure of PQ for radical cure of hypnozoites.

974

QUININE TREATMENT IN PREGNANT WOMEN WITH MALARIA-HIV CO-INFECTION: PILOT OBSERVATIONAL STUDY

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Pregnant women bear the greatest burden of malaria-HIV co-infection. Quinine continues to be important in the treatment of uncomplicated malaria in early pregnancy and severe malaria. Previous studies suggest that HIV infection and interaction between antimalarial and antiretroviral drugs may negatively influence antimalarial pharmacokinetics and treatment outcomes. We conducted a pilot clinical study to assess quinine pharmacokinetics in Malian pregnant women co-infected with acute *falciparum* malaria and HIV. All ten participants had PCR-corrected 28-day adequate clinical and parasitologic responses. Adverse events were frequent. One of the ten women who reported taking nevirapine-based antiretroviral therapy showed no measurable concentrations of nevirapine in her plasma. Plasma concentration of both total and free (unbound) quinine were lower, and the major active metabolite 3-hydroxyquinine higher, in pregnant women who had a stable plasma concentration of nevirapine than in the participant who did not. Quinine trough concentrations were below the recommended therapeutic range of 5-15 mg/L in 50% of the women who provided plasma samples. The study findings suggest that chronic administration of nevirapine-based antiretroviral therapy may negatively influence quinine pharmacokinetics, and co-administration of these drugs may also increase the risk of drug toxicity. Further research is warranted to understand the impact of HIV as a chronic disease and its long term antiretroviral therapy on the treatment of acute malaria.

975

INTERMITTENT PARASITE CLEARANCE IN SCHOOLCHILDREN: IMPACT ON COGNITION IN AN AREA OF HIGHLY SEASONAL TRANSMISSION

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Malaria control has usually focused on those at most risk of malaria-related mortality (pregnant women and children under five). Yet recent studies show that older school-age children can also benefit from malaria control, with potential gains for both health and education. Mali introduced universal coverage of nets with community-wide distributions starting in 2011. Whilst successful in achieving high levels of coverage, 80% of schoolchildren remained infected at the end of the malaria transmission season in Nov 2011. This calls for additional control measures in this age group. One potential supplementary strategy is intermittent parasite clearance in schools (in which a treatment dose is given irrespective of infection status) with the aim to improve educational performance by reducing malaria-related anaemia and improving cognitive function among schoolchildren. This approach is particularly suited to areas of seasonal transmission where a single annual treatment can be given at the end of the transmission season. A cluster-randomized controlled trial was conducted in 80 primary schools in Sikasso, Mali where the majority of schoolchildren already slept under insecticide-treated nets. Children in intervention schools received a single treatment dose at the end of Nov 2011; administered in school by teachers over three consecutive days. Parasite clearance was associated with dramatic reductions in malaria parasitaemia and gametocyte carriage at follow-up in Feb 2012 in intervention compared to control schools. This effect was sustained until May 2012, the beginning of the next transmission season. Malaria parasite clearance was also associated with a significant decrease in anaemia (OR=0.56, 95% CI 0.39 to 0.78, p=0.001), and increase in sustained attention (p<0.001). Findings from the full battery of cognitive and educational tests will be presented, and discussed in relation to the growing body of evidence on the impact of asymptomatic malaria infection on cognitive performance in schoolchildren, and control strategies in areas of highly seasonal transmission.

976

PLASMODIUM FALCIPARUM PFS47 GENE MEDIATES EVASION OF THE MOSQUITO IMMUNE SYSTEM

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Some *Plasmodium falciparum* lines are able to infect the refractory *Anopheles gambiae* L3-5 strain by evading its complement-like immune system. A Quantitative Trait *Loci* (QTL) mapping was carried out to identify the *P. falciparum* gene(s) that allow evasion of the *A. gambiae* immune system. The gene mapping was done in a cross between *P. falciparum* GB4 that successfully infects the A.*gambiae* L3-5 strain, and the 7G8 line which is eliminated in *A.gambiae* L3-5 by melanotic encapsulation. QTL analysis identified one main significant locus in chromosome 13 that is associated with the phenotype; this locus was confirmed independently by linkage group selection in individual oocysts. Candidate genes were selected for detailed genetic analysis based on gene expression differences and sequence polymorphisms between the parental lines. Knocking out Pfs47 in *P. falciparum* NF54, a line that infects *A. gambiae* L3-5, results in a line that is melanized in *A. gambiae* L3-5. This melanization is dependent on the mosquito complement-like system and can be rescued by genetic complementation with the wild type gene. The Pfs47 protein, a member of the 6-cys protein family, was found to be present in the surface of ookinetes. Taken together, all the evidence indicates that Pfs47 mediates *P. falciparum* evasion of the *A. gambiae* immune system. Pfs47 may be important to understand adaptation of the parasite to different Anopheline mosquitos around the world, and could be a target for transmission blocking strategies.

977

MULTIPLE MECHANISMS OF LATE-PHASE IMMUNITY LIMIT *PLASMODIUM* DEVELOPMENT IN THE MOSQUITO *ANOPHELES GAMBIAE*

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Mosquitoes of the genus *Anopheles* serve as the obligate vectors of the malaria parasite *Plasmodium*. During its development within the mosquito host, several factors and developmental bottlenecks limit parasite success, including two distinct phases of the mosquito innate immune response. Emerging evidence suggests that parasite numbers are largely influenced by an "early-phase" that targets ookinetes as they reach the basal lamina of the mosquito midgut through the exposure to complement-like components of the hemolymph and a "late-phase" response that limits oocyst survival through the production of nitric oxide mediated by the STAT pathway. Recently, we have identified a novel LITAF-like transcription factor (LL3) in *Anopheles gambiae* that is an important component of the mosquito response to *Plasmodium*. Following LL3-silencing, oocyst numbers are significantly increased and evidence suggests that LL3 is involved in the "late-phase" response by limiting oocyst survival. Experiments to determine the relationship between the "late-phase" response for LL3 and those previously described for the STAT pathway imply that the LL3 and STAT phenotypes occur through independent, yet closely related mechanisms involving hemocyte function. Current experiments aim to further dissect LL3 function to address a critical gap in our knowledge of the mosquito late-phase immune response and the mechanisms that limit oocyst survival in its mosquito host.

978

CHARACTERIZATION OF THE TARGET OF IVERMECTIN, THE GLUTAMATE-GATED CHLORIDE CHANNEL, IN *ANOPHELES GAMBIAE* AND AS A TARGET OF A MOSQUITOCIDAL VACCINE

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The use of insecticide-treated nets and indoor residual insecticides targeting adult mosquito vectors is a key element in malaria control programs. However, the spread of insecticide-resistant mosquitoes has countered these efforts to control vector populations. Recently, the use of ivermectin mass drug administration has been shown to kill *Anopheles gambiae* mosquitoes and disrupt malaria transmission in the field. We cloned the molecular target of ivermectin from *An. gambiae*, the glutamate-gated chloride channel (AgGluCl), and characterized its protein expression and activity to glutamate and ivermectin. Cloning revealed four AgGluCl splice isoforms with heterogeneity between isoforms found in the N-terminal extracellular domain and the intracellular loop region. AgGluCl expression was observed throughout the mosquito nervous systems including neuropil associated with multiple sensory and motor systems. Activity was measured using two-electrode voltage clamp on *Xenopus laevis* oocytes expressing AgGluCl to glutamate and ivermectin. Ivermectin was shown to induce non-persistent AgGluCl activity and does not potentiate glutamate responses, which is unique among GluCls. An alternative strategy to chemical insecticides for parasite transmission control is the development of mosquitocidal and transmission-blocking vaccines. By feeding *An. gambiae* blood meals containing polyclonal IgG targeting AgGluCl, we were able to kill the majority of blood fed mosquitoes (LC_{50}: 2.82mg/mL). We show that blood meals containing AgGluCl IgG block AgGluCl activity, either through AgGluCl antagonism or internalization of AgGluCl from the membrane surface.

979

GENETIC MANIPULATION OF *ANOPHELES STEPHENSI* IMMUNITY TO INCREASE *PLASMODIUM FALCIPARUM* SALIVARY GLAND SPOROZOITE INFECTION LEVELS

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Sanaria's technology platform generates live, aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites (PfSPZ) that can be administered as a highly protective malaria vaccine. PfSPZ are produced using *Anopheles stephensi* mosquitoes as bioreactors. The capacity of *A. stephensi* to support *Plasmodium* sporogonic development to fully infective salivary gland stage sporozoites is dictated by the activities of several known components of the mosquito's innate immune system. In order to increase the yield of sporozoites per mosquito, thereby enhancing PfSPZ manufacturing efficiency, we are genetically modifying mosquitoes to reduce activity of the IMD signaling pathway and the downstream effector molecule LRIM1. Two approaches are being taken: 1) overexpression of CASPAR, a negative regulator of the IMD pathway; and 2) employing novel technologies to reduce expression of the LRIM1 gene by introducing hairpin-DNA transgenic constructs encoding LRIM1-specific siRNAs. We will present our progress generating transgenic lines of *A. stephensi* and the levels of *P. falciparum* sporogonic infection achieved in transgenic mosquitoes relative to appropriate control mosquitoes.

980

WAAL GENE OF *ENTEROBACTER SP.* AG1 FROM THE MOSQUITO *ANOPHELES GAMBIAE*: ROLES IN LIPOPOLYSACCHARIDE (LPS) BIOSYNTHESIS AND OXIDATIVE STRESS DEFENSE

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Enterobacter bacteria are core residents in the gut of mosquito *Anopheles gambiae*, which are dominant in the gut microbial community after blood feeding. We isolated a strain of Enterobacter from the G3 colony of *An. gambiae*, and sequenced its genome. In vitro, the bacterium showed strong tolerance to paraquat, an oxidative stress inducer. In order to identify genes that are involved in the protection against oxidative stress, a mutant library of *Enterobacter sp.* Ag1 was generated using transposon-mediated mutagenesis. One mutant was identified, in which the waaL gene was disrupted. WaaL gene encodes O antigen ligase, which ligates the O antigen to the core-lipid A during LPS synthesis. The mutant had deficient LPS structure on the LPS PAGE gel. The LPS structure was restored in the cells that were complemented with a waaL gene containing plasmid. The mutant grows normally in LB culture with no difference from the wildtype. However, the mutant lost its tolerance to 6mM paraquat. In addition, the mutant was more sensitive to H2O2 stress. Taken together, the data suggest that waaL gene is required for LPS biosynthesis, and is involved in the protection against oxidative stress. LPS is the major component of the cell wall in Gram negative bacteria. The mutant can be used as a model to study the roles of LPS in colonization of the gut and bacteria-host interactions.

981

AEDES AEGYPTI LABORATORY ADAPTATION LEADS TO GLOBAL TRANSCRIPTOMIC DOWN-REGULATION AND DENGUE VECTOR CAPACITY CHANGES

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Current knowledge of dengue-mosquito interactions is derived from studies that utilized laboratory-adapted mosquito strains maintained in an insectary environment for decades. A better understanding of the genetic and transcriptomic changes that occur in field-derived mosquito strains over the course of laboratory adaptation would provide valuable insight into how mosquitoes adapt to a specific environment and how this adaptation influences vector capacity. After being maintained in a laboratory environment for generations, dengue virus susceptibility of the field-derived mosquito strains changed over time. Microarray analyses of the laboratory-adapted mosquitoes revealed a general down-regulation of the transcript abundance of genes involved in processes such as metabolism, transcription, translation, and immunity, which provides the evidence of a genetic bottleneck due to the laboratory environment. Our result revealed that specific rearing conditions can promote transcript profile changes that may both directly and indirectly affect mosquito susceptibility to dengue virus. Functional assays of these genes provide further insight to the individual contribution of these potential dengue virus host-factors.

982

AEDES MCINTOSHI: GENETIC DIVERSITY AND MAGNITUDE OF RIFT VALLEY FEVER IN KENYA

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The complex interplay of virus-host-vector cycle driven by climate change has been attributed to the spread and sporadic outbreak of many arboviral diseases such as Rift Valley fever (RVF). However, the genetics of vectors in conditioning their maintenance and spread is least appreciated despite variation which may be evident in the pattern of outbreak occurrence. We used both mitochondrial and nuclear markers to characterize the genetic structure of *Aedes mcintoshi* populations, a key vector of Rift Valley fever (RVF) virus, from 14 sites including virus-endemic/free areas and epidemic prone areas of Kenya using Neighbor-joining trees, Bayesian inference, median joining network and analysis of molecular variance (AMOVA). Both gene *loci* indicated 4 supported genetic lineages with significant genetic diversity among them across the study areas. The lineages display geographic restriction reflecting the magnitude of RVF in Kenya possibly influenced by prevailing environmental conditions in these locations. Broadly for both markers, lineage I was restricted to Central, Rift Valley and Western areas and lineages III and IV restricted to localities in North Eastern Kenya. However within North Eastern Kenya, the epicenter of RVF epidemics in Kenya, these two lineages (III and IV) occur in sympatry in most of the localities sampled but overall low mean evolutionary divergence estimates between the lineages suggest a variant or cryptic species in that region. Furthermore, disproportionate abundance of these lineages in these localities and their presence/absence may drive differential transmission and outbreak patterns of the disease in different communities of North Eastern Province of Kenya.

983

TIME-VARYING, SEROTYPE-SPECIFIC FORCE OF INFECTION ESTIMATES FOR DENGUE VIRUS USING LONGITUDINAL SEROLOGICAL DATA

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The utility of disease models for planning public health interventions and policy relies on accurate estimates of key transmission parameters. These parameters are often estimated as constants in time, not always because of the perceived veracity of that assumption, but often as a consequence of data constraints such as the lack of long-term, longitudinal infection data. One such parameter, the force of infection, is the per-capita risk of a susceptible individual becoming infected. The force of infection captures the fundamental dynamics of transmission and is crucial to gauging necessary control efforts as well as informing vaccine deployment. Dengue virus (DENV) is a multi-serotype, mosquito-borne, viral infection that causes an estimated 390 million human infections each year. Here, we describe a new spline-based fitting procedure developed to compute time-

varying serotype-specific estimates of the force of infection using a 12-year longitudinal DENV dataset from Iquitos, Peru. The dataset contained information on 14,335 individuals (47,121 blood samples) and 23,989 serotype-specific DENV infections. Of these, 3,621 occurred during the study period, which enabled estimation of when the infections took place. Depending on year and serotype, yearly force of infection varied from 0 to 0.33. We identified periods of synchronization between serotypes, but there was no consistent pattern in which serotypes experienced simultaneous outbreaks. As an extension of our approach we calculated time-varying serotype-specific estimates of the basic reproductive number (R0) for DENV, which varied from less than 1 to 5.43, depending on year and serotype. Our results provide important new insights into DENV transmission dynamics that will inform implementation of vector management strategies and deployment of vaccines, when they become available.

984

FINE-SCALE HUMAN MOVEMENT: THEORY, DATA AND IMPLICATIONS FOR DENGUE VIRUS TRANSMISSION

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From traveling on an airplane to commuting for work to visiting neighbors, human movement plays a major role in the spread of infectious diseases at a variety of scales. Models of fine-scale movement (e.g., within a city) and data to inform them have been lacking for resource-poor areas, which are afflicted by a multitude of diseases and where movement patterns are likely to depart substantially from those in developed temperate areas. Moreover, aspects of movement most relevant to the transmission of a mosquito-borne disease such as dengue fever have received little explicit consideration in existing models. We developed a new model for simulating individual human movement and fit it to interview data collected from 149 residents of the city of Iquitos, Peru. We then simulated city-wide movement networks for Iquitos and compared the properties of those networks to movement networks in other settings. Finally, we applied human movements simulated by our model to a recently developed framework for modeling mosquito-borne disease transmission. Our model of individual human movement has five distinct components that together describe variation in how individuals allocate their time across a dynamic set of locations: the number of locations visited, what types of locations are visited, where those locations are, and how often and for how long individuals visit each location. Fitting the model to data revealed that movement differs among locations of different types, that distance from home strongly influences where people go and how often and for how long they visit, and that some variation in movement patterns can be explained by individual attributes, such as age or sex. Network properties of simulated movement across the city differ in several ways from comparable movement networks from cities in developed countries, and they are also sensitive to assumptions about secondary network structures not captured by our model; e.g., social relationships. Combining simulated movements with information about spatial variation in mosquito densities and mosquito movement, we show that disease invasion probabilities and threshold criteria for disease persistence change precipitously as fine-scale variation is homogenized at increasingly aggregated scales.

985

SYMPTOMATIC VERSUS INAPPARENT OUTCOME IN REPEAT DENGUE VIRUS INFECTIONS IS INFLUENCED BY THE TIME INTERVAL BETWEEN INFECTIONS AND STUDY YEAR

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Four dengue virus serotypes (DENV1-4) circulate globally, causing more human illness than any other arthropod-borne virus. Dengue can present as a range of clinical manifestations from undifferentiated fever to classic Dengue Fever to severe, life-threatening syndromes. However, most DENV infections are inapparent. Yet, little is known about determinants of inapparent versus symptomatic DENV infection outcome. Here, we analyzed over 2,000 DENV infections from 2004 to 2011 in a prospective pediatric cohort study in Managua, Nicaragua. Symptomatic cases were captured at the study health center, and paired healthy annual samples were examined on a yearly basis using serological methods to identify inapparent DENV infections. Overall, inapparent and symptomatic DENV infections were equally distributed by sex. The mean age of infection was 1.2 years higher for symptomatic DENV infections as compared to inapparent infections. Although inapparent versus symptomatic outcome did not differ by infection number (first, second or third/post-second DENV infections), substantial variation in the proportion of symptomatic DENV infections among all DENV infections was observed across study years. In participants with repeat DENV infections, the time interval between a first inapparent DENV infection and a second inapparent infection was significantly shorter than the interval between a first inapparent and a second symptomatic infection. This difference was not observed in subsequent infections. This result was confirmed using two different serological techniques that measure total anti-DENV antibodies and serotype-specific neutralizing antibodies, respectively. Taken together, these findings show that, in this study, age, study year and time interval between consecutive DENV infections influence inapparent versus symptomatic infection outcome, while sex and infection number had no significant effect. Moreover, these results suggest that the window of cross-protection induced by a first infection with DENV against a second symptomatic infection is approximately 2 years. These findings are important for modeling dengue epidemics and development of vaccines.

986

PHYLOGENETIC EVIDENCE FOR THE EMERGENCE OF A NORTH AMERICAN LINEAGE OF DENGUE VIRUS SUBTYPE 1

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Dengue, one of the most important re-emerging tropical diseases, is caused by four dengue virus subtypes (DENV-1-4) .Transmission in previously non-epidemic areas is documented every year. The proliferation of urban centers, frequent international travel, and considerable climatic changes, probably contributes to the increased distribution of DENV, as evidenced by emergence of new phylogenetic lineages. In 2010, Nuevo Leon, Mexico, a region that had reported only limited dengue cases since 2005, reported 2271 laboratory-positive cases, with 99.6% of the isolates being DENV-1. Concomitantly, Key West, Florida experienced

endemic DENV-1 transmission in 2009 and 2010. These occurrences indicate the establishment of endemic DENV transmission in new areas of North America. We performed in-depth sequence analyses on the envelope gene from 81 DENV-1 isolates from the Americas including 17 sequences obtained from clinical cases during the 2010 Nuevo Leon outbreak and 14 sequences from cases during the 2009-2010 epidemic in Key West. Maximum likelihood and Bayesian phylogenetic analyses identified the emergence of a new DENV-1 lineage currently propagating in North America. This North American lineage emerged from the Central American lineage of the American-African genotype evolving in a short period of time following a northbound transmission trajectory. The recent DENV-1 emergence into non-endemic areas seems to have driven the divergence of new monophyletic sublineages associated with particular epidemics in Nuevo Leon and Key West. Vector density, geographic, and climatic variables were found to be associated with the increase in dengue incidence facilitating the propagation of the mosquito vector and the virus within susceptible populations. *In situ* microevolution of this emerging lineage has been identified confirming the establishment of this lineage in new regions of North America. Our findings reveal the emergence of a new lineage of DENV-1 circulating in North America. The transmission of this virus in new areas seems to have driven the evolution of this lineage. This dynamic of DENV evolution demonstrates the ability of the virus to follow the movement of the human host, and increased transmission in new areas.

987

OUTCOMES OF INFANTS WHOSE MOTHERS HAD SYMPTOMATIC DENGUE INFECTION DURING PREGNANCY: A RETROSPECTIVE COHORT STUDY

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Data on dengue infection and its possible effects on the developing fetus are scarce. Existing case reports have suggested higher rates of preterm birth and low birth weight for infants whose mothers had dengue during pregnancy, but many questions remain since, to our knowledge, no epidemiologic investigation on the infant outcomes of mothers with dengue during pregnancy has been done. We conducted a retrospective cohort study in St. Laurent du Maroni, French Guiana, examining the poor birth outcomes of preterm birth (PTB) and low birthweight (LBW) among infants whose mothers had symptomatic dengue during pregnancy. Conditional logistic regression was used to match each of the 86 exposed infants to the three unexposed births immediately following to create a stratum. Due to changes in reporting of miscarriages over time, a sensitivity analysis including and excluding infants at various gestational ages was conducted. The three categories used in the sensitivity analysis were; all infants regardless of gestational age and their strata, only infants 17 weeks of gestational age or older and their strata, and only infants 22 weeks of age or older and their strata. Odds ratios were adjusted for maternal age, maternal ethnicity, maternal gravidity, interpregnancy interval and maternal anemia. After adjustment, PTB demonstrated an increased risk in the dengue exposed group (aOR 22 weeks: 1.41 (0.39, 5.20), aOR 17 weeks: 1.89 (0.61, 5.87), aOR all infants: 3.34 (1.13, 9.89)). Adjusted results for LBW were similar, demonstrating an increased risk in the exposed group (aOR 22 weeks: 1.43 (0.56, 3.70), aOR 17 weeks: 1.67 (0.71, 3.93), aOR All infants: 2.23 (1.01, 4.90)). The results of this study indicate that symptomatic dengue infection in pregnancy may increase the risk of PTB and LBW in infants.

988

COST-EFFECTIVENESS OF A NOVEL TECHNOLOGY FOR DENGUE PREVENTION IN CHILDREN: INSECTICIDE-IMPREGNATED SCHOOL UNIFORMS

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Children carry a larger proportion of the disease burden of dengue in endemic countries and are more likely to experience the complications of the disease, including dengue haemorrhagic fever (DHF), compared to adults. Prevention is key to reducing morbidity and mortality because no specific curative treatment exists for the disease. Vector control using adulticides and larvacides has shown some but very limited impact on dengue incidence. The implementation of vector control activities is particularly challenging in urban and peri-urban areas, where the vector is well-established and thriving on proximity to humans. The search for novel, acceptable and affordable methods of vector control is ongoing. Because dengue vectors bite primarily during the day, schools_where children spend most of their day_are an ideal setting for dengue prevention and control. A community-based controlled trial is currently underway in eastern Thailand to assess the impact of impregnated school uniforms on the incidence of dengue in school-aged children. While awaiting the results of the trial, a recent modeling study has shown that "the use of insecticide-impregnated uniforms has an efficacy varying from around 6% in the most pessimistic scenario to 55% in the most optimistic scenarios simulated." Following standard guidelines of economic analyses, we will develop a decision analytical model to evaluate the potential health and economic value of this new intervention from the societal perspective, using data from the published literature specific to Thailand. The model will simulate the incidence of dengue fever and DHF, survival, disability adjusted life years, and costs in a hypothetical cohort of children receiving the intervention and will facilitate a comparison against scenario of no intervention. The outcome of this analysis will be expressed as a ratio of incremental costs to incremental health outcomes of the intervention. We will perform sensitivity analysis of key variables and assumptions.

989

DENGUE-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN PUERTO RICO

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Hemophagocytic lymphohistiocytosis (HLH) is a rare, potentially fatal disorder characterized by hyperinflammation due to uncontrolled proliferation of activated lymphocytes, resulting in prolonged fever, pancytopenia, jaundice, and hepatosplenomegaly. HLH can be familial or acquired, the latter being the result of malignancy or infection. Dengue-associated HLH (dengue-HLH) has been described in 26 case reports since 1966, but has not been previously recognized in Puerto Rico. In December 2012, CDC Dengue Branch was notified of several dengue-HLH cases at two San Juan pediatric hospitals. An investigation was conducted to: 1)

determine the incidence of HLH since 2008; and 2) determine the infecting agent(s) associated with HLH cases. Medical records were queried to identify patients with findings compatible with HLH. To date, 480 records have been reviewed and 18 patients identified that met accepted criteria for HLH. Sixteen (84%) HLH cases had diagnostic evidence of DENV infection by IgM ELISA (44%) or PCR (56%): dengue virus types -1and -4 were detected. There was one fatal dengue-HLH case (case-fatality rate [CFR]: 5.5%). Dengue-HLH cases ranged in age from 0.2 -16 years, 50% were infants, and all resided in northern Puerto Rico. Among children aged 0-16 years, the annual incidence of dengue-HLH cases was 1.8 per 100,000 population. The median serum ferritin value was 22,524 µg/L (range: 754-522,000 µg/L) in dengue-HLH cases. Hemophagocytosis was evident in bone marrow aspirates of six of 11 (55%) dengue-HLH cases for which testing was performed. Median hospital stay was 26 days (range: 8-81 days). Only one hospital consistently used immunosuppressive therapy for suspected or confirmed HLH cases. We are conducting a case-control study to identify risk factors for developing dengue-HLH and to determine why infants were predominantly affected. Some symptoms of HLH may also be seen in patients with severe dengue, potentially resulting in under-recognition. Physicians in dengue endemic areas should be made aware of HLH.

990

CD47 DEFICIENCY PROTECTS AGAINST *PLASMODIUM BERGHEI* ANKA AND *P. CHABAUDI* AS INFECTION

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Malaria pathogenesis is dependent upon the invasion and maturation of the malaria parasite within host red blood cells (RBCs). Protection against severe and fatal malaria is associated with RBC polymorphisms where structural and functional defects limit invasion and growth of the parasite. CD47 engagement by macrophage SIRPα inhibits phagocytosis and prevents RBC uptake. We have recently shown that CD47 levels are lower on *Plasmodium falciparum*-parasitized RBCs and consequently increases the phagocytosis of parasitized RBCs *in vitro*. In the present study we examine whether blockade of CD47-SIRPα confers protection *in vivo* using two different murine models of severe malaria. C57BL/6 mice lacking CD47 (Cd47-/-) and congenic controls (Cd47+/+) were inoculated intraperitoneally with 106 *P. berghei* ANKA (PbA) or with 106 *P. chabaudi chabaudi* AS (PccAS) parasitized RBCs and were monitored twice daily for 21 days. All congenic control mice infected with PbA died within 6-9 days post-infection with symptoms of experimental cerebral malaria. In contrast, mice with CD47 deficiency displayed profoundly lower parasite burdens ($P < 0.0001$) and had significantly improved survival ($P < 0.0001$) compared with their Cd47+/+ expressing littermates. Similarly, compared to congenic control mice that develop high *parasitemia* when infected with PccAS, Cd47-/- mice were completely refractory to PccAS infection. In conclusion, these results indicate an important role for CD47/SIRPα blockade in protection in murine models of experimental severe anemia and cerebral malaria.

991

T-BET PREVENTS DEVELOPMENT OF PROTECTIVE IMMUNITY BUT MAY SUPPRESS T CELL DEATH INDUCED BY *PLASMODIUM YOELII* 17XNL INFECTION

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$CD4^+$ T cells are important mediators of malaria immunity. However, the effect of differentiation programs that lead to the generation of $CD4^+$ T cell subsets and influence other aspects of host immunological networks are poorly understood. We used mice deficient for T-bet, the master regulator of Th1 $CD4^+$ T cell differentiation, to examine the effect of Th1 $CD4^+$ T cells on immune protection to nonlethal murine malaria *Plasmodium yoelii* 17XNL. T-bet-deficient C57BL/6 mice had significantly lower (2.9 fold) *parasitemia* compared to wildtype C57BL/6 mice indicating that the T-bet transcription factor hinders the formation of protective immunity in this murine model. Analysis of the B cell compartment of adaptive immunity demonstrated that T-bet-deficient mice produced abrogated levels of IgG2a and abundant levels of IgG1 antibodies suggesting that altered regulation of antibody isotype switching in the absence of T-bet may be responsible for the enhanced immune protection observed in these mice. Remarkably, absence of T-bet was also associated with a transient but significant loss (2.5 fold) of T cells during the ascending phase of *parasitemia* (day 8) followed by limited expansion of T cells during the descending phase of *parasitemia* (day 14) suggesting that T-bet may suppress malarial antigen induced T cell death. While T-bet-deficient mice had significantly fewer T cells compared to WT mice, there was no observed loss of IFN-γ^+CD4^+ and IFN-γ^+CD8^+ T cells indicating that IFN-γ producing T cells are preferentially expanded in T-bet-deficient mice. These results are further corroborated by a 6.7 fold increase in serum IFN-γ in T-bet-deficient mice during the clearance phase (day 22) of infection. Lastly, T-bet-deficient mice produce comparatively greater numbers of $Foxp3^+CD25^+$ regulatory $CD4^+$ T cells. Future studies are directed at determining whether this excess of $Foxp3^+CD25^+$ regulatory $CD4^+$ T cells is responsible for the early contraction and limited expansion of T cells observed in T-bet-deficient mice and elucidating the mechanism of T-bet mediated suppression of malarial antigen induced T cell death.

992

MAPPING *LOCI* ASSOCIATED WITH PARASITE CLEARANCE AND VIRULENCE USING MURINE MODELS OF MALARIA

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Malaria is the most deadly parasitic disease of mankind, killing more than half a million children each year. A better understanding of the molecular basis governing disease pathology will help reduce mortality and morbidity. Murine malaria parasites have served as important models for studying host-parasite interaction and disease pathogenesis. Here we have genetically crossed two *Plasmodium yoelii* parasites that have different growth characteristics and cause different host mortality, to identify novel parasite factors that can influence host response and disease severity. Fourty-six recombinant progeny were cloned and identified from the cross after typing the progeny with microsatellite markers. Using a new assembly of the *P. yoelii* genome and Next Generation Sequencing data of multiple isolates we generated, we designed a genotyping microarray containing approximately 11,000 single nucleotide polymorphisms between the two parental strains for quick and accurate assessment of progeny genotypes. Quantitative trait *loci* analysis (QTL) using disease phenotypes and genotypes from recombinant progeny identified two chromosomal *loci* on Chromosome 7 and 13 that are linked to parasite growth and disease severity. Further analysis of the *loci* revealed additive interactions between the *loci* identified. We are in the process of validating candidate genes in the *loci* that could potentially be explored for vaccine development.

993

SHIFTED CLEARANCE OF ONCE-INFECTED ERYTHROCYTES CONTRIBUTES TO POST ARTESUNATE NON INFECTIOUS DELAYED ANEMIA (PANDA) IN SEVERE MALARIA PATIENTS CURED WITH INTRAVENOUS ARTESUNATE

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Intravenous artesunate is now the recommended first-line therapy for severe malaria worldwide. Recently, an original pattern of anemia that occurs abruptly days to weeks after complete parasite clearance has been reported in European travellers cured by artesunate. The mechanism of this post-treatment complication is unknown. Through an optimized national surveillance program, we collected clinical and laboratory data from 125 *Plasmodium falciparum*-infected travelers treated with iv artesunate. Of 72 patients followed beyond 8 days post-admission (D8), 35 (48%) had a conventional "rising" pattern of hemoglobin concentration without hemolysis after D8, 17 (24%) had a typical abrupt "recurring" anemia pattern Post-Artesunate Non-infectious Delayed Anaemia (PANDA) defined by a greater than 10% drop in hemoglobin concentration and/or rise in LDH concentration occurring after D8, and 15 (21%) had a "persisting", stable anemia/hemolysis pattern. The kinetics of circulating once-infected erythrocytes (from which dead parasites had been removed by the spleen-specific pitting process) was determined in 12 patients. The concentration of once-infected erythrocytes peaked before D8. It was higher than 60% of initial parasitaemia in the 7 patients with subsequent PANDA but lower than 40% of initial *parasitemia* in the 5 patients with other patterns of anemia. During artesunate treatment intra-erythrocytic rings are rapidly killed but host erythrocytes remain intact and return to the circulation after the parasite remnants have been expelled by the spleen-specific pitting process. Pitting clears artesunate-exposed parasites without destroying host erythrocytes but spared once-infected erythrocytes are eventually removed from the circulation 2 to 3 weeks later. This shifted clearance contributes to the peculiar kinetics of recurring anemia in patients cured by artesunate. Early quantification of once-infected erythrocytes may help predict the risk of PANDA in this context.

994

ERYTHROCYTE INVASION RECEPTOR PREFERENCES OF *PLASMODIUM FALCIPARUM* ISOLATES IN GHANAIAN CHILDREN

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Clinical manifestations of *Plasmodium falciparum* infection are caused by invasion of erythrocytes by the malaria parasite, a process which is mediated by multiple receptor-ligand interactions. Antibodies against some parasite ligands have been shown to significantly inhibit parasite growth *in vitro*, demonstrating that these interactions may be good targets for the development of an effective blood stage vaccine. This study was aimed at investigating the erythrocyte receptors used by *P. falciparum* isolates in Ghana. *P. falciparum* isolates were collected from children aged 2-14 years attending hospitals in three ecologically distinct zones in Ghana: Accra, Kintampo and Navrongo. Erythrocyte invasion assays were performed to test the ability of the parasites to invade erythrocytes treated with neuraminidase, trypsin and chymotrypsin, which selectively remove receptors from the erythrocyte surface. In addition, antibodies against two recently identified receptors, basigin and complement receptor 1 (CR1) were used to determine the dependence of the isolates on these pathways. Two to four assays were performed on each isolate. All 16 field isolates tested so far were capable of invading neuraminidase-treated erythrocytes, with invasion efficiencies of 40-80% relative to untreated erythrocytes, indicating that these parasites had sialic acid-independent invasion phenotypes. Invasion of trypsin or chymotrypsin-treated erythrocytes varied between 20-60% relative to untreated erythrocytes representing the contributions of glycophorins A, B, and C. Furthermore, for nearly all the parasites tested, anti-CR1 antibodies significantly inhibited invasion of neuraminidase-treated erythrocytes, confirming the role of CR1 as the major sialic acid-independent receptor for *P. falciparum*. Additional isolates are being tested and results from about 50 parasites will be presented.

995

IMPAIRED ENDOTHELIAL AND MICROVASCULAR FUNCTION IN *VIVAX* MALARIA IN PROPORTION TO DISEASE SEVERITY

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Plasmodium vivax is now recognised as causing severe disease, including acute lung injury, shock, acute kidney injury and severe anemia. The pathogenesis of disease however is poorly understood. In contrast to *falciparum* malaria, parasite biomass is lower in *vivax* malaria due to a predilection for reticulocytes, and parasite sequestration, the hallmark of severe *falciparum* malaria, does not occur to a significant degree. Endothelial and microvascular function are impaired in severe *falciparum* malaria, with each contributing to impaired organ perfusion, but have not been evaluated in *vivax* malaria. We measured endothelial function using reactive hyperemia-peripheral arterial tonometry (RH-PAT), and microvascular reactivity using thenar muscle near-infrared resonance spectroscopy, in patients with severe (n=8) and non-severe (n=30) *vivax* malaria, and compared results with severe (n=18) and non-severe (n=72)

falciparum malaria and 79 healthy controls (HC). Endothelial function was impaired in proportion to P.*vivax* disease severity (median RH-PAT index: severe *P. vivax* (1.49, IQR 1.37-1.88), non-severe *P. vivax* (1.73, IQR 1.46-2.05) vs HC (1.97, IQR 1.64-2.27; ANOVA p=0.024), with function in severe *vivax* malaria at least as low as in severe *falciparum* malaria (median 1.5, IQR 1.26-1.75). Endothelial function recovered by day 3 in severe *vivax* malaria (2.04, IQR 1.76-2.24; p=0.046). Median microvascular reactivity (StO2 recovery; U/sec) was lower in patients with severe *vivax* malaria (5.44, IQR 3.62-6.01) compared to patients with non-severe *vivax* malaria (6.98, IQR 5.79-7.52; p=0.006) and controls (6.34, IQR 5.21-7.03; p=0.027). Endothelial and microvascular function are impaired in severe *vivax* malaria, with endothelial dysfunction comparable to severe *falciparum* malaria. Endothelial and microvascular dysfunction may underlie pathogenesis of severe *vivax* malaria.

996

DNA SEQUENCE REARRANGEMENTS IN THE *PLASMODIUM VIVAX* GENOME

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Plasmodium vivax remains a major public health problem that threatens half of the world's population. The emergence of novel mechanisms of invasion and the rise of drug resistance highlights the need for continued research on this parasite. Recent whole genome sequencing of *P. vivax* from field and monkey-adapted isolates by our lab and others have provided a framework for expanding our knowledge of the molecular polymorphism and global diversity of this parasite. In addition to identifying single nucleotide polymorphisms, whole genome sequencing can also be used to characterize DNA sequence rearrangements. Our analyses reveal very large telomeric deletions, evidence of ectopic recombination in MSP gene clusters as well as deletions and duplications involving well-characterized genes. These rearrangements include the deletion of a gene encoding a Phist protein in the Belem strain, the deletion of the reticulocyte-binding-protein-2-like gene in many strains, and a tandem duplication of ~10kb containing the entire Duffy-binding protein gene in several Malagasy field isolates. The duplication of PvDBP in Malagasy strains is of potential further interest as we, and others have shown that *P. vivax* is capable of infecting red cells and causing malaria in Duffy-negative people. A global survey of *P. vivax* infections revealed that this duplication is common in Madagascar but rare or absent elsewhere. The highly conserved nature of the duplicated sequence suggests this rearrangement occurred in a relatively recent evolutionary time frame. These genomic changes reveal evidence of past or on-going *P. vivax* evolution and suggest avenues by which the parasite can increase its genetic diversity and survival capacity.

997

A NEW MOUSE MODEL FOR FEMALE GENITAL SCHISTOSOMIASIS

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Female genital schistosomiasis (FGS) is caused by *Schistosoma haematobium* eggs deposited in the human female reproductive tract by adult *S. haematobium* worms. Although FGS causes pelvic pain, vaginal bleeding, disfigurement, and infertility, it also increases the risks of contracting sexually transmitted diseases such as HIV. The associated mechanisms remain unclear due to the lack of a tractable animal model. To model FGS in mice, we injected *S. haematobium* eggs into the posterior vaginal walls of female mice. This resulted in reproducible, synchronous vaginal granuloma development within 2 weeks post-egg injection and lasting for at least 8 weeks after injection. Flow cytometric analyses of vaginal tissues revealed the presence of T cells with variable expression of the HIV target molecules CXCR4 and CCR5. Granulomata contained CD11b+F4/80+ cells (macrophages and eosinophils) as well as CXCR4+MerTK+ macrophages. Strikingly, vaginal wall-injected mice featured significant urinary frequency despite the egg-injected posterior vagina being anatomically distant from the bladder. We speculate that mammals have evolved a bladder overactivity response to deposition of schistosome eggs in the vagina since egg deposition in the bladder often accompanies FGS. This response would facilitate expulsion of bladder-deposited eggs in order to minimize sequelae from chronic egg-induced bladder inflammation. Ongoing studies will ascertain the biologic basis of this vagina-bladder reflex, as well as characterize the mechanisms by which HIV target cells are recruited to vaginal granulomata.

998

PERSISTENT ORGANOMEGALY ASSOCIATED WITH SCHISTOSOMIASIS AND CORRELATES OF ABNORMAL LIVER PATTERN IN YOUNG KENYAN CHILDREN

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Schistosoma mansoni infection is a major cause of liver fibrosis and other morbidity in adults. However, morbidity in young children, currently excluded from mass treatment campaigns, is less defined. We previously reported results of a cross-sectional study, nested within a treatment trial in Mbita, Kenya, of a convenience sample of 201 children under 7 years. *S. mansoni* infection was associated with hepatomegaly and splenomegaly, but not image pattern B (IPB), a liver pattern seen on ultrasound, considered a possible intermediate stage in development of fibrosis due to *S. mansoni*. We now report impact of praziquantel (PZQ) treatment on infection and morbidity in these children, a mean of 3.2 (range 1.6-4.0) months post-treatment and 7 months post-baseline. Data included stool exam for current *S. mansoni* infection, blood tests for malaria by smear and rapid diagnostic test (RDT), and baseline infection status. We also used the WHO Niamey ultrasound protocol to stage hepatosplenic damage from *S. mansoni*, including left-lobe hepatomegaly, splenomegaly and liver image pattern. Longitudinal analyses controlled for age, sex, and RDT result; adjusted cross-sectional models also included clustering by village and current *S. mansoni* infection. Among 137 children with longitudinal organometry data who received PZQ, prevalence of *S. mansoni* infection decreased from 42 to 33%. Heavy-intensity infection (greater than 400 eggs per gram) decreased from 10 to 2%. Malaria prevalence increased from 2 to 17% by smear, and was 36% by RDT. Prevalence of IPB increased from 14 to 21%; hepatomegaly, 54 to 68%; splenomegaly, 30 to 44%. Treatment was not associated with improved organomegaly regardless of initial infection status. Among 146 children with post-treatment organometry, current *S. mansoni* infection was associated with increased hepatomegaly on univariable (prevalence ratio [PR]=1.4; p<.01) and adjusted (adjusted PR [aPR]=1.3; p<.01) analysis but not splenomegaly. Among 180 ultrasounded children, IPB was associated with malaria by RDT on univariable (PR=2.0; p<.01) and adjusted (aPR=2.0, p<.01) analysis but not with current *S. mansoni*. A mean of 3.2 months post-treatment, *S. mansoni* infection was common, and was still associated with hepatomegaly in this young cohort in the setting of interval increases in malaria and organomegaly. IPB may not be pathognomonic for *S. mansoni* infection in young children, but may be caused by malaria.

999

AN *EX VIVO* MODEL FOR STUDYING THE EARLIEST PHASE OF HEPATIC SCHISTOSOMIASIS

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To investigate the earliest hepatic events associated with the deposition of schistosome eggs, we have established a novel technique, involving the culturing of naïve murine thin liver slices (250 µm) in conjunction with exposure to soluble egg antigens. This system has allowed us to identify the transcriptional events that contribute to the initiation of the subsequent granulomatous response. Initially tissue, without parasite antigen exposure, was analysed for general histological changes, the presence of liver enzymes indicative of hepato-toxicity and finally RNA quality. All of these parameters indicated the fidelity of the tissue, and the sterility over 48 hour period were maintained. Next to build on these research tools, we have employed microarray analysis of the tissue with and without parasite egg antigen, in order to allow us to follow the dynamics of antigen presentation, inflammation and general hepatotoxicity, which represent the initial phases that will lead to pathology. Findings from this *ex vivo* approach, are currently being integrated with data from our previously *in vivo* whole organ studies with *Schistosoma japonicum*. We eventually aim to identify the contribution of hepatic and systemic immune cell types in the host transcriptional response to egg deposition and the resulting granuloma formation in host liver.

1000

SCHISTOSOMIASIS JAPONICA DURING PREGNANCY IS ASSOCIATED WITH ELEVATED ENDOTOXIN LEVELS IN MATERNAL AND PLACENTAL COMPARTMENTS

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Schistosomiasis affects approximately 40 million women of reproductive age, and chronic infection has been linked to elevated levels of endotoxin in circulation. Whether this is also true in pregnancy complicated with schistosomiasis has not been evaluated. In this study, we measured endotoxin levels in maternal peripheral (32 wks gestation), placental and newborn plasma collected from a cohort of 133 women in Leyte, The Philippines. Birth outcomes, cord blood, placental biopsy and placental blood were collected at delivery; endotoxin levels were measured in all plasma samples. Placental biopsies were evaluated for a number of histopathological outcomes related to placental inflammation. After adjusting for confounders, endotoxin levels in pregnant women with schistosomiasis were higher in the maternal and placental plasma than in uninfected women (1.3-fold, $P = 0.03$ and 2.4-fold, $P < 0.001$, respectively). Premature birth and acute chorioamnionitis were associated with elevated levels of endotoxin in the placental plasma (2.5-fold higher endotoxin in premature births, $P = 0.01$, 2.0-fold in acute chorioamnionitis, $P = 0.04$). A host of pro-inflammatory cytokines such as IL-6 (7.1-fold, $P < 0.001$), TNF-α (6.1-fold, $P <0.001$), IFN-γ (1.8-fold, P 0.05), IL-1 (14.7-fold, $P <0.001$), and CRP (2.8-fold, $P <0.001$) were elevated in maternal plasma among women with endotoxin levels in the highest tertile of the distribution. Additionally, some anti-inflammatory cytokines including IL-10 (2.1-fold, $P <0.001$), IL-5 (2.2-fold, $P =0.01$), and IL-13 (2.1-fold, $P <0.001$) were elevated in the placental plasma of these subjects. We have previously shown that schistosomiasis during pregnancy can elicit a pro-inflammatory cytokine response in placental, maternal and cord blood. Herein, we report for the first time that *S. japonicum* infection at delivery is also associated with elevated levels of endotoxin in maternal and placental plasma. These data suggest additional mechanisms by which schistosomiasis negatively impacts the maternal-fetal dyad.

1001

HELMINTH-INDUCED IL-4 ABOLISHES INKT CELL-MEDIATED CLEARANCE OF *BACTERIURIA*

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Infection with *Schistosoma haematobium*, the cause of urogenital schistosomiasis, is a risk factor for bacterial urinary tract co-infection. This co-infection worsens the sequelae of urogenital schistosomiasis, including hematuria, dysuria, and risk of bladder cancer. Despite the impact of these infections, it is unknown how co-infection by *S. haematobium* and bacterial uropathogens impairs host clearance of bacterial UTI. Many helminth infections, including schistosomiasis, induce host leukocytes to secrete IL-4. It is unknown whether IL-4 impairs bacterial clearance in schistosome-bacterial co-infections. Another ill-defined but key facet of anti-bacterial immunity is the role of iNKT cells. To study the mechanisms of *S. haematobium*-bacterial uropathogen co-infections, we combined the first tractable model of urogenital schistosomiasis with an established mouse model of bacterial UTI. This model recapitulates human co-infection, since a single bladder exposure to *S. haematobium* eggs triggers IL-4 production and renders a mouse strain susceptible to bacterial UTI when it otherwise is resistant (BALB/c). During co-infection, bladders are infiltrated by fewer iNKT cells than during bacterial UTI alone. Moreover, co-infection results in lower CD1d expression in bladder dendritic cells and lower levels of IFN-γ in bladder iNKT cells on a per-cell basis. We have found that three distinct conditions can restore the baseline resistance of BALB/c mice to bacterial UTI despite prior exposure to *S. haematobium* eggs: 1) antibody neutralization of IL-4, 2) genetic deficiency of IL-4 receptor-α signaling, and 3) exogenous gly*coli*pid antigen-induced activation of iNKT cells. We hypothesize that *S. haematobium* egg-induced IL-4 reduces CD1d expression by antigen-presenting cells, which dampens iNKT cell-derived, IFN-γ-mediated clearance of bacteriuria. Continuing work will test this hypothesis, and may enable iNKT cell-based therapies as alternatives to antibiotic treatment of UTI more broadly.

1002

SCHISTOSOMIASIS *JAPONICA* SOLUBLE EGG ANTIGENS INTERFERE WITH DIFFERENTIATION AND INVASION OF PLACENTAL TROPHOBLAST CELLS

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Schistosomiasis represents a significant disease burden in endemic regions. We have previously shown that schistosomiasis during pregnancy results in a pro-inflammatory cytokine response detectable in maternal, placental, and cord blood as well as increased pathological signs of placental inflammation. Our previous data suggest this response is at least partly due to altered cytokine production by the trophoblast cells of the placenta. In addition to immune balance, trophoblasts are responsible for the majority of placental functions, including invasion of the uterine lining and hormone production for the maintenance of pregnancy. Herein, we have expanded our previous data to examine the effect of schistosome soluble egg antigens (SEA) on specific aspects of trophoblast function, including differentiation, invasion and hormone production. Primary cytotrophoblasts were collected at term from uninfected North American placentas and placed in culture for 5d, during which time they spontaneously differentiate to syncytiotrophoblast, the cell layer responsible for nutrient/gas/waste exchange and hormone production. Real-time qPCR for syncytin, a marker of trophoblast differentiation, showed a decrease in mRNA levels in cells exposed to SEA for the final

24h of the culture period. Progesterone production in this model system also tended to be lower in trophoblasts exposed to SEA. In addition, we used the HTR8/SVneo cell line as a model of extravillous trophoblast with invasive characteristics. Treatment of HTR8 cells with SEA for 24h resulted in a significant drop in the number of cells that invaded through a matrigel-coated membrane. Interestingly, there was no difference in either model system in the degree of TUNEL staining, suggesting SEA does not cause apoptosis in trophoblast cells. These data suggest that SEA interferes with the differentiation and function of trophoblast cells, and thus may predispose affected pregnancies to poor placentation and consequent birth outcomes.

1003

MATERNAL SCHISTOSOMIASIS *JAPONICA* INDUCES A FIBROGENIC RESPONSE IN NEONATES

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The global burden of schistosomiasis is significant, with fibrosis one of the primary morbidities associated with the disease. Although the etiology and endpoints of fibrosis are thought to occur locally by surrounding eggs trapped in the liver, many molecules associated with fibrosis can be measured in the blood of infected individuals. We have previously shown that schistosomiasis during pregnancy results in a pro-inflammatory cytokine response in the cord blood of the affected neonate. In this study, we extended these findings to include a large panel of fibrogenic markers. A multiplex bead-based assay (FibroPlex v2) was developed in our laboratory to measure the levels of 35 mediators, effect modifiers, and outcomes associated with fibrosis. Cord blood from a cohort of 109 neonates born to mothers residing in an *Schistosoma japonicum* endemic area was assessed for all 35 of these fibrosis-related molecules. After adjusting for potential confounders, ten distinct pro-fibrotic mediators were significantly higher ($P < 0.05$) in the cord blood samples from infants whose mothers were infected with schistosomiasis during gestation (N=56) compared to infants born to uninfected mothers (N=53). These included IGF-1 (1.4 fold higher in neonates from infected mothers), TGF-β1 (2 fold higher), CTGF (2.7 fold higher), PICP (1.6 fold higher), ICTP (1.5 fold higher), collagen VI (1.3 fold higher), desmosine (1.8 fold higher), MMP-2 (4.4 fold higher), TIMP-1 (1.6-fold higher), and TIMP-4 (1.5 fold higher). Our data demonstrate that maternal schistosomiasis is sufficient to elicit a 'fibrogenic signature' in the cord blood of the neonate. As the first report of fibrosis-associated molecules altered in the newborn of infected mothers, these data have broad implications for the health of the developing fetus *in utero*, as well as after birth and into adulthood.

1004

DEEP PROFILING OF THE NOVEL INTERMEDIATE-SIZE NONCODING RNAS IN INTRAERYTHROCYTIC *PLASMODIUM FALCIPARUM*

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Intermediate-size noncoding RNAs (is-ncRNAs) have been shown to play important regulatory roles in the development of several eukaryotic organisms. However, is-ncRNAs have not been thoroughly explored in *Plasmodium falciparum*, the most virulent malaria parasite that affects humans. In order to understand the whole profile of is-ncRNAs in *P. falciparum*, we performed a systematic identification of novel is-ncRNAs in intraerythrocytic *P. falciparum* 3D7 using Illumina/Solexa paired-end sequencing of an is-ncRNA-specific library. A total of 1,159 novel is-ncRNAs, including antisense, intergenic, and intronic is-ncRNAs were identified. Bioinformatics analyses indicated that the intergenic is-ncRNAs were the least conserved among eight different *Plasmodium* species, and antisense is-ncRNAs were more conserved than their sense counterparts. Thirty-six novel sno/scaRNAs were predicted, seven potential novel classes of is-ncRNAs were discovered by clustering analysis, and two novel internal motifs of intergenic is-ncRNAs were identified. The expression of selected novel is-ncRNAs was confirmed by RT-PCR and northern blotting assays. An obvious difference in the novel is-ncRNA expression profiles of the early and late intraerythrocytic developmental stages of the parasite was observed by Realtime PCR, suggesting the expression of the novel is-ncRNAs is regulated tightly in the parasite. Many of the novel is-ncRNAs showed a higher expression in the early stage than in the late stage, implying their role in building the components and structures used in later stage. The expression levels of four antisense RNAs in our study were shown to be co-regulated with that of their *cis*-encoded sense RNAs, suggesting that these antisense RNAs are involved in the regulation of gene expression in the parasite. This study provides indispensible information to the whole noncoding transcriptome of the parasite and will help further function study of the novel is-ncRNAs in during the intraerythrocytic development of *P. falciparum*.

1005

ON THE ORIGIN OF ANTIGEN SEQUENCE DIVERSITY IN *PLASMODIUM FALCIPARUM*: VAR GENES UNDERGO FREQUENT MITOTIC RECOMBINATION

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Plasmodium falciparum is a unicellular parasite responsible for severe human malaria. The parasite divides asexually within infected red blood cells (iRBCs), and coats the erythrocyte surface with highly polymorphic proteins in the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family. These are encoded by ~60 var genes per genome, and play an important role in immune evasion through antigenic variation and parasite sequestration to the microvasculature. We investigated how diversity is generated in var genes using a clonal dilution system to isolate single iRBCs, followed by whole genome next generation DNA sequencing of the expanded populations derived from these single cells. We cultured three *P. falciparum* strains from diverse geographic origins over 4-8 months, sub-cloning every 4-8 weeks, and sequenced 106 samples at high coverage. Single Nucleotide Polymorphisms (SNPs) were distributed throughout the genome at a rate of ~2x10^-9 SNP/generation/nucleotide, suggesting that a point mutational process does not contribute to var gene diversity. In contrast, all of the 41 structural variants identified in coding regions occurred within var genes, and produced chimeric sequences through gene conversions and duplications. Recombination occurred within 'identity blocks' (IBs) - runs of 4-48bp (average: 15bp) of identical sequences between the recombining var genes, though no conserved motif was observed. The ~80bp surrounding the IB mid-point were more homologous than the background var gene level, and the resulting chimeric var genes were kept in frame with no SNPs or InDels. These data suggest that homology-dependent DNA repair is responsible for var gene recombination in mitosis, and we speculate that this process has evolved to increase var gene sequence diversity and produce novel PfEMP1 antigens. This has direct implications for the parasite's survival in semi-immune malaria patients.

1006

EXPRESSION VARIATION IN *PLASMODIUM FALCIPARUM*: IMPLICATIONS FOR PARASITE ADAPTATION

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Phenotypic plasticity is crucial in biological systems because it allows populations to adapt to constantly changing environments. Phenotypic heterogeneity within populations of genetically identical cells is an important but often overlooked source of phenotypic plasticity. Most investigations currently focus on the average of quantitative traits; however, several recent studies in model organisms have demonstrated that variation in expression is an adaptive genetically controlled phenotype that can be repeatedly measured. Thus, we anticipate expression variation will be important for adaptation in *Plasmodium falciparum*. Even small variations in transcriptional abundance among the millions of parasitized red blood cells within a single malaria infection may provide a selective advantage to a subset of the parasite population, thereby enhancing parasite fitness. To study the innate biological expression variation in the malaria parasite, we compared the gene expression variance within five sub-cloned lines of HB3, Dd2, and select progeny from the cross. For each parasite sub-clone, genome-wide gene expression levels were obtained using a custom designed high density exon array. We observed that parasite sub-clones from the same line have diverging gene expression profiles when measured at the same developmental stage and environmental conditions. For example, gene expression variation within HB3 sub-clones was much higher than within Dd2 sub-clones. This could be related to the strong selection bottlenecks that Dd2 has undergone, including drug selection pressure under mefloquine and chloroquine. It could also indicate that drug resistant parasites are more susceptible to rapidly changing environments. The distinct gene expression variation within the sub-clones of the parental lines opens the possibility that their progeny will show varying levels of transcriptional plasticity. Therefore, our extended study could lead to the discovery of genetic determinants of this plasticity, a potentially important factor in the adaptability of the parasite to drugs.

1007

FUNCTIONAL ANALYSES OF SPOROZOITE RHOPTRY PROTEINS BY STAGE-SPECIFIC GENE SILENCING SYSTEM IN *PLASMODIUM BERGHEI*

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Malaria transmission to mammalians is initiated by sporozoites inoculation into the skin via mosquito bite. Sporozoites migrate to the liver through the blood vessel then invade hepatocytes within parasitophorous vacuole, where they develop into thousands of erythrocyte invasive forms. Rhoptry is one of the apical organelles observed only in sporozoites and merozoites. Recently, it has been suggested that rhoptry proteins are localized to the tight junction and have important roles during merozoite invasion of erythrocyte. Here, we focused on sporozoite rhoptry proteins to elucidate the molecular mechanisms of sporozoite infectious ability. Since it was shown that 8 proteins are expressed and localized to rhoptries both in merozoites and sporozoites, we intend to analyze their functions during sporozoite infection of target cells by reverse genetics. Therefore, we generated stage specific gene silencing transgenic parasites for each gene by replacing the endogenous promoter to the merozoite-specific promoter. In vivo and *in vitro* infectivity analyses of mutant sporozoites reveal that some proteins are required for salivary gland invasion and others for liver invasion. This is the first reverse genetic evidence to show that rhoptry proteins are involved in target cell infection.

1008

ESSENTIAL ROLE FOR A KINASE IN *PLASMODIUM FALCIPARUM* GAMETOCYTE PRODUCTION

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Malaria transmission from an infected human host to a mosquito vector relies on the production of male and female gametocytes. Despite the crucial role that these sexual stage parasites have in the malaria lifecycle, little is known about the triggers or molecular mechanisms that mediate sexual development. We have previously shown that *Plasmodium falciparum* gametocyte development 1 gene (*Pfgdv1*) is critical for early sexual differentiation; however other genes are likely to also be required for gametocyte production. Candidates include kinases that could be involved in the initiation or propagation of the cascade of gene activation that is associated with sexual development. Here we report on the localisation of PFB0665w, a predicted serine/threonine kinase that has a transcription profile similar to *Pfgdv1,* in sexually committed rings and stage I-IV gametocytes, using immunofluorescence microscopy of transgenic parasites expressing the HA-tagged enzyme. Targeted disruption of *PFB0665w* eliminated gametocyte production, suggesting the gene is critical for sexual differentiation. This is consistent with our previous results showing that *PFB0665w* is likely dispensable for asexual blood stage *P. falciparum* infection. Reverse-transcription quantitative PCR confirmed expression of *Pfgdv1* in the *PFB0665w* minus cell line, indicating that it is not required for *Pfdgv1* expression. Based on our data, we suggest PFB0665w is essential for gametocyte production and that it functions downstream of Pfgdv1 in the gametocytogenesis cycle. This role together with its continuous expression throughout sexual differentiation potentially makes PFB0665w an attractive target for a transmission-blocking strategy to eliminate malaria. We are currently investigating if the kinase is also expressed in schizonts whose progeny is pre-committed to gametocytogenesis, as is the case for the NIMA-related kinase Pfnek-4.

1009

THE ROLE OF GLUTATHIONE BIOSYNTHESIS IN MODULATING DRUG RESPONSE IN *PLASMODIUM FALCIPARUM*

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Glutathione, a tripeptide (γ-glutamylcysteinyl-glycine, GSH) is a major reduced thiol in *Plasmodium* with important functions as a redox buffer and as a cofactor for detoxifying enzymes. GSH is synthesized in *Plasmodium* in two steps: the first and the rate-limiting step is catalyzed by gamma-glutamyl cysteine synthetase (γ-GCS) followed by glutathione synthetase (GS). The genes encoding these proteins are believed to be essential in *Plasmodium falciparum*. Modulation of γ-GCS and the antioxidant network have been recently reported to be associated with artemisinin and dihydroartemisinin resistance in *P. yoelii* and *P. falciparum* and interestingly; in a recent genome-wide association study (GWAS) we observed associations between the non-synonymous single nucleotide polymorphisms (SNPs) K173I in γ-GCS and I376L in GS and parasite response to dihydroartemisinin. We also sequenced the gene encoding γ-GCS and observed a variable repetitive motif of (Y/C)QS(N/D)LQQQ, repeating in tandem between 5X-15X in 60 strains of *P. falciparum* worldwide. This length polymorphism was not associated to *in vitro* culture adaptation, since the repetitive motifs were identical in paired *ex vivo* and culture adapted parasites. While in 22% of these strains, including 3D7, the sequence motif was present only once, we observed a significant association between length polymorphism of γ-GCS and parasite response to lumefantrine, halofantrine, and mefloquine in 25 Senegalese clinical isolates, suggesting that length polymorphism in this gene may have a functional role. To understand the importance of the different variants

of γ-GCS and GS, we overexpressed HA-tagged versions of the wild-type and mutant γ-GCS and GS alleles in 3D7 parasites. Western blot analysis confirmed the expression of HA-tagged γ-GCS and GS across the life cycle, with protein expression peaking at early trophozoite stage. We observed that overexpression of γ-GCS conferred resistance to L-buthionine sulfoximine (BSO), an irreversible inhibitor of γ-GCS. We are currently studying the levels of GSH, accumulation of reactive oxygen species (ROS) and the responses of these over-expressors to several antimalarials and to agents that induce oxidative stress and testing the hypothesis if specific antimalarial agents work by modulating the GSH bio-synthetic pathway.

1010

PROTEIN HOMEOSTASIS AS A NOVEL TARGET FOR ANTIMALARIAL THERAPY: A STUDY OF THE PROLYL-TRNA SYNTHETASE INHIBITOR HALOFUGINONE IN *PLASMODIUM FALCIPARUM*

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Many current anti-malarial drugs work within the same biological pathways leading to shared resistance mechanism. We have taken the methodology of chemogenomics to identify potential antimalarials that target novel pathways. Understanding the anti-plasmodial mechanism of halofuginone (HFG), a febrifuginone analogue, informs our understanding of parasite biology and directs the future creation of novel therapies. To interrogate mechanism, we selected parasites that are resistant to halofuginone and then used whole genome sequencing to identify the causative mutation (SNPs) and developed high resolution melting (HRM) genotyping assays to follow up those most promising. We found two nonsynonomous mutations in the active site of the cytoplasmic prolyl-tRNA synthetase (*Pf*cPRS) in independent selections and have validated PfcPRS as the target of Halofuginone using a heterologous *S. cerevisiae* model, recombinantly expressed protein, and *Plasmodium falciparum* reverse genetics. Now, we have further characterized the role of the halofuginone and related stresses on amino acid starvation and other mechanisms of protein homeostasis in *P. falciparum*. Treatment of parasites with halofuginone and febrifugine results in increased phosphorylation of a *P. falciparum* eIF2α analogue. Overall, these results demonstrate halofuginone-induced proline starvation via an interaction with *Pf*cPRS leads to translational inhibition. Thus we posit that amino acid supply and aminoacyl tRNA synthetases are a new and promising target for chemotherapeutic intervention.

1011

FUNCTIONAL ROLES FOR C5A AND C5AR, BUT NOT C5L2, IN THE PATHOGENESIS OF HUMAN AND EXPERIMENTAL CEREBRAL MALARIA

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The host immune response plays an important role in the onset and progression of cerebral malaria (CM). The complement system is an essential component of the innate immune response to malaria, and its activation generates the anaphylatoxin, C5a. To test the hypothesis that C5a signaling contributes to the pathogenesis of CM, we examined plasma levels of C5a in children with CM vs. uncomplicated malaria (UM), and investigated a causal role for the C5a receptors, C5aR and C5L2, in a mouse model of experimental CM (ECM) induced by *Plasmodium berghei* ANKA (PbA) infection. In a nested case-control study of Ugandan children, the median levels of C5a at presentation were significantly higher in children with CM versus those with UM (43.7 vs. 22.4 ng/mL; $p < 0.001$). In the ECM model, *C5aR-/-* mice displayed significantly improved survival compared to their wild-type (WT) counterparts ($p=0.004$); whereas *C5L2-/-* mice showed no difference in survival from WT mice. Improved survival in *C5aR-/-* mice was associated with reduced levels of pro-inflammatory cytokines TNF, IFN and MCP-1. Furthermore, endothelial quiescence and integrity in the brain were enhanced as demonstrated by increased levels of angiopoietin-1, decreased levels of angiopoietin-2 and soluble ICAM-1, and decreased Evans blue extravasation. These findings demonstrate that C5a is dysregulated in human CM and contributes to the pathogenesis of ECM via C5aR-dependent inflammation and endothelial dysfunction.

1012

RAPID, PITTING-INDEPENDENT CLEARANCE OF *PLASMODIUM FALCIPARUM* IN IMMUNE MALIAN CHILDREN TREATED WITH ARTESUNATE FOR UNCOMPLICATED MALARIA

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In patients with *Plasmodium falciparum* malaria, artemisinin-induced clearance of infected red blood cells (iRBCs) is faster than clearance induced by other antimalarial drugs. This difference has been attributed to "pitting," a spleen-specific process whereby parasites altered by artemisinins are expelled from their host RBCs. After pitting, once-infected RBCs (O-iRBCs) are released intact into the circulation. Parasite clearance time (PCT) and peak levels of O-iRBCs in the circulation were analyzed in French travelers with severe malaria, and in artemisinin-treated Malian children with uncomplicated malaria. O-iRBCs peaked at 95% and 25% of initial *parasitemia* in artesunate- and quinine-treated travelers, thus confirming the major role of pitting in artemisinin-induced parasite clearance in non-immune patients. In Malian children, mean PCT was short (20 hours) and significantly shorter in 9- to 13-year-old children than in 0.5- to 4-year-old children (14 vs. 26 hours, $p=0.0001$). As expected, peak O-iRBC levels were significantly lower in older than in younger children (27.2% vs. 93.9%) suggesting that the very short PCT in older children was in part unrelated to pitting rate. In Malian children, the proportion of iRBCs recognized by autologous IgG ex vivo correlated significantly with PCT ($r=-0.501$, $p=0.0006$) and peak O-iRBC levels ($r=-0.420$, $p=0.0033$). Lag phases on parasite clearance curves were shorter in older children and infants, suggesting the existence of an immune-dependent clearance mechanism occurring faster than pitting in infants with passively-acquired humoral immunity from their mother and in older children with multiple previous exposures to *P. falciparum*. Mechanisms of *P. falciparum* clearance in artemisinin-treated patients may be more diverse than previously thought.

1013

MALARIA INFECTION DEPLETES HEPATIC DDAH1, A REGULATOR OF ENDOTHELIAL NITRIC OXIDE SYNTHESIS

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Impaired endothelial nitric oxide (NO) synthesis is associated with the clinical severity and risk of death from malaria. Endothelial NO synthesis is dependent upon the blood levels of arginine, the substrate for NO synthesis, and asymmetric dimethylarginine (ADMA), a potent inhibitor of NO synthesis. Homeostasis of the arginine to ADMA ratio is maintained by hepatic dimethylarginine dimethylaminohydrolase-1 (DDAH1), an enzyme that metabolizes ADMA at a rate inversely proportional to arginine concentration. A genome-wide association study recently identified a polymorphism in DDAH1 to be associated with susceptibility to severe malaria in Gambian children. We hypothesized that malaria infection causes DDAH1 dysfunction, dysregulation of the arginine to ADMA ratio, and impaired NO synthesis. To test this hypothesis, we infected C57Bl/6 mice with *Plasmodium berghei* ANKA, an established model of severe malaria. DDAH1 protein concentration was determined by quantitative analysis of Western blots of liver homogenates. Tissue and plasma concentrations of ADMA and arginine were determined by HPLC. Nitrite, an indicator of NO synthesis, was measured by gas-phase chemiluminescent assay. Data represent 3 independent experiments. Six days after inoculation with *P. berghei*, protein levels of DDAH1 fell by 55% compared to uninfected controls ($p < 0.0001$). Concurrently, the proportion of arginine to ADMA fell from 118 ± 28 in control animals to 87 ± 22 in infected animals ($p < 0.001$). Blood nitrite levels fell from 0.53 ± 0.1 uM in control animals to 0.36 ± 0.1 uM in infected animals, consistent with impaired NO synthesis ($p < 0.01$). This experimental model of severe malaria recapitulated the decreased arginine to ADMA ratio and decreased nitrite levels observed in West African children with severe malaria. Analysis of hepatic tissue revealed loss of DDAH1 protein to be a potential mechanism explaining the dysregulation of ADMA metabolism and the impairment of NO synthesis that occurs during severe malaria. These findings encourage further investigation into the naturally occurring human genetic variants of DDAH1 that determine susceptibility to severe malaria in African children.

1014

ANTI-SELF ANTIBODIES AGAINST PHOSPHATIDYLSERINE INDUCE ANEMIA IN MALARIA

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Severe malarial anemia is one of the leading causes of mortality in malaria. *Plasmodium* invades red blood cells (RBCs) to mature and reproduce, but this represents only a minimal loss compared to the massive elimination of uninfected RBCs, which contributes decisively to anemia in malaria . Using a mouse model, we have found that *Plasmodium* infection induces the generation of $CD4^+$ T cell-dependent anti-self antibodies that bind to the surface of infected and uninfected RBCs from infected animals, but not from control uninfected mice. Phosphatidylserine (PS), which is exposed on the surface of a high fraction of uninfected RBCs during malaria, is recognized by these antibodies, facilitating their phagocytosis by macrophages. When anti-self anti-PS antibodies are transferred into *Plasmodium* infected mice, a significant increase in anemia is observed, which is not found when they are transferred into control uninfected animals or when irrelevant antibodies are transferred. Conversely, blocking of PS in infected mice through the injection of annexin V results in a faster recovery from anemia. These findings indicate that autoimmune antibodies induced by malaria, recognize PS on the surface of uninfected erythrocytes and mediate their clearance, contributing to anemia. Inhibition of this pathway may be potentially exploited for treating malarial anemia.

1015

INVESTIGATING THE ANGIOPOIETIN-TIE2 PATHWAY AS A THERAPEUTIC TARGET TO IMPROVE SURVIVAL FOLLOWING EXPERIMENTAL LIFE-THREATENING *PLASMODIUM* CHALLENGE

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Cerebral malaria (CM) pathogenesis is associated with endothelial activation and loss of blood brain barrier integrity. The angiopoietin (Ang)-Tie2 signalling pathway is a key regulator of endothelial function. Alterations in the angiogenic balance, specifically increased Ang-2 relative to Ang-1, has been associated with poor clinical outcome in CM. It remains unclear whether the Ang/Tie2 pathway is causally involved in CM pathogenesis. We hypothesize that dysregulation in angiopoietins contributes to ECM pathogenesis, and interventions to maintain Tie2 activation may promote endothelial stability, prevent deleterious alterations to the BBB and improve outcome following *Plasmodium* infection. Using the murine model of *Plasmodium berghei* ANKA (PbA)-induced experimental CM (ECM), we show that altered protein and mRNA levels of angiopoietins are associated with disease severity, similar to observations in human populations, and directly precede the loss of vascular integrity in the brain and the onset of severe neurological symptoms, such as seizures and paralysis. Therapeutic intervention to maintain Tie2 activation (e.g. Adenoviral expression of Ang-1) significantly improved survival in ECM-susceptible C57Bl/6 mice compared to empty adenoviral vector controls and vehicle controls ($p=0.001$, logrank test) and prevented ECM-induced neurological impairment. The survival benefit was further increased when therapeutic Ang1 was delivered in combination with a sub-curative dose of the anti-parasitic drug artesunate ($p=0.0039$) compared to the benefit of artesunate alone. Conversely, genetic deletion using a conditional Cre/loxP system showed that moderately resistant Balb/c mice have significantly worsened disease outcome in the absence of normal Ang/Tie2 interaction ($p=0.01$), further supporting a critical role of angiogenic-Tie2 signaling during ECM. Overall, these findings underscore the contribution of the angiopoietin-Tie2 pathway to the pathobiology of ECM and show that adjunctive treatment strategies based on promoting endothelial quiescence improve survival in ECM.

1016

CHARACTERIZATION OF THE DIFFERENTIAL INVASION OF *PLASMODIUM FALCIPARUM* INTO YOUNG AND OLD RBCS

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The blood stage of a malaria infection is responsible for all disease, and *Plasmodium falciparum* is the most pathogenic of all the malaria species to infect humans. *P. falciparum* is capable of infecting all host RBCs regardless of age, however infection rate of young RBCs is greater than old RBCs. RBC physiology changes during the course of the 120 day RBC lifespan, culminating in the signals required to trigger clearance of senescent RBCs from circulation. As RBCs age cell volume, enzymatic activity, deformability, membrane protein abundance and sialation decrease while cell density, osmotic fragility, and oxidative damage increase. We show that the abundance of important RBC *P. falciparum* invasion ligands Basinigin (CD147) and CR-1 (CD35) decrease with increasing RBC age. Furthermore, we utilize a novel three color invasion assay to investigate the

contribution of (i) RBC *P. falciparum* invasion ligands (ii) sialation of RBC surface proteins, and (iii) membrane rigidity on the differential invasion of *P. falciparum* into young and old RBCs. We show that decreased density of RBC invasion ligands on old RBCs does not contribute to the decreased invasion rate of *P. falciparum* into old RBCs, but that differences in RBC membrane rigidity and membrane protein sialation do influence the differential invasion of young and old RBCs by *P. falciparum.* Our investigation emphasizes the dynamic nature of the host RBC population and the contribution that changing RBC physiology has on *P. falciparum* infection.

1017

USE OF A NOVEL FLUORESCENT-LABELED SINGLE CHAIN ANTIBODY FOR PARATRANSGENIC CONTROL OF LEISHMANIASIS

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Leishmaniasis is caused by protozoan parasites of the genus *Leishmania*, and is transmitted by the bite of an infected Phlebotomine sand fly. This disease is a leading cause of morbidity in the world, with close to 12 million people infected in over 80 countries worldwide. Transmission control is heavily dependent on the use of chemical pesticides. However, environmental toxicity, adverse effects on human health and the emergence of insect resistance have greatly undermined their efficacy. We have reported on a paratransgenic strategy to control vectorial transmission of this neglected tropical disease in previous work. Here we describe the development of a novel effector molecule for use in this paratransgenic approach. The monoclonal antibody B72.3 binds to sialyl-(le)a glycan, and was demonstrated to bind with specificity to the surface of *L. donovani, L. mexicana* and *L. major* promastigotes. We have generated a recombinant single chain antibody (scFv) of B72.3, and have demonstrated that it binds with specificity to several *Leishmania spp.* We have subsequently replaced the linker region between the VH and VL domains with monomeric red fluorescent protein mRFP from Discosoma, generating a fluorescent REDantibody. There are numerous reports citing the release of reactive oxygen species (ROS) following illumination of photosensitizer proteins such as mRFP. By linking the mRFP to the B72.3 scFv, we have targeted this ROS release to invading *Leishmania* promastigotes. We hypothesize that exposure of the paratransgenic sand fly to the intense light conditions in their native environments would lead to the release of ROS by the B72.3 REDantibody. These molecules are expected to result in membrane damage, leading to decrease parasite viability in the paratransgenic sand fly. We have validated that this modified version of the B72.3 scFv binds with specificity to three *Leishmania spp.* In preliminary studies, we were able to detect ROS release from the B72.3 REDantibody following exposure to a halogen light source. The ability of the ROS release following light exposure to decrease parasite load *in vitro* are currently underway utilizing mid-gut binding assays.

1018

LEISHMANIA INFLUENCE DIFFERENTIAL MICRORNA EXPRESSION PROFILES WITH IMMUNOLOGICALLY CRITICAL TARGET TRANSCRIPT NETWORKS AMONG HOST CELLS

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Leishmania donovani (*Ld*) and *L. major* (*Lm*) are vector-borne intracellular protozoan parasites and two Old World causative agents of visceral and cutaneous leishmaniasis respectively. These pathogens avoid innate immune destruction when parasitizing host macrophages (MP) or dendritic cells (DC), in part, by eliciting unique host cell type-specific transcriptional profiles. We explored the role of translational regulation by host microRNAs (miRNAs) in a *Leishmania* species specific manner via RNAseq, microarray, and quantitative proteomics analyses. Total mature miRNA expression profiles were explored through next generation sequencing of small RNAs (<30nt) isolated from a time course of DC and MP *in situ* infections with *Lm* and *Ld* separately. Expression profiles of large coding RNAs was assessed using microarray; and protein expression was determined via quantitative mass spectrometry of matched donor samples. Results of integrative multi-dimensional dataset analyses revealed differential miRNA expression profiles which were host cell type and infecting parasite species specific. Most miRNAs were downregulated across infection conditions compared to uninfected controls. Among DC infected with *Ld*, a small selective miRNA group was upregulated. Correlative target prediction analysis identified negative TGFβ pathway regulator transcripts as the primary targets of *Ld* upregulated miRNAs. miRNA promoter analysis also revealed docking sites for TGFβ induced transcription factors. Functional studies are underway to confirm these target predictions and assess the potential for TGFβ pathway dysregulation by *Ld* induced miRNAs as a permissive mechanism for *Leishmania* parasite survival. This study serves as a pilot for further investigations into the differentially selective functions of host cell miRNAs during *Ld* and *Lm* infection.

1019

CHARACTERIZATION OF CROSS PROTECTION BY GENETICALLY MODIFIED LIVE ATTENUATED *LEISHMANIA DONOVANI* PARASITES AGAINST CUTANEOUS LEISHMANIASIS CAUSED BY *L. MEXICANA*

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Leishmaniasis causes significant morbidity and mortality worldwide and there is no vaccine against this disease. Previously we showed that genetically modified live attenuated *L. donovani* parasite cell lines ($Cen^{-/-}$ and $p27^{-/-}$) induce a strong cellular immune response providing protection against visceral leishmaniasis in mice. In the current study, we show that both these cell lines induce a strong pro-inflammatory response and stimulate the nitric oxide production in bone marrow derived mouse dendritic cells in contrast to wild type parasites. Upon challenge with wild type *L. mexicana*, mice immunized either for short (6 weeks) or long (8 months) periods showed significantly smaller lesions and lower parasite burden than naïve mice. Immunized and challenged mice showed a well organized collagen deposition indicating wound healing, whereas naïve mice had a dispersed and sparse collagen bundle after challenge indicated a growing lesion. Immuno-histochemical analysis of mice ear lesions from immunized and challenged mice showed significant influx of macrophages, MHCII expressing cells and nitric oxide producing cells. Further, after virulent challenge, the presence of de-granulated mast cells in lesions of non-immune mice, but not in immunized mice, confirmed parasite control by immunization. Cytokine analysis of the *L. donovani* antigen stimulated splenocyte culture supernatants from live attenuated parasite immunized, *L. mexicana* challenged mice revealed induction of both secreted IFNγ and IL4, suggesting a systemic mixed Th1 and Th2 immune response against the immunizing agent. However, *L. mexicana* antigen stimulated lymph node cell culture supernatants from immunized challenged mice revealed higher IFNγ secretion and suppression of IL10, IL4, IL5, IL13 and IL6 compared to non-immunized challenged mice suggesting attenuated *L. donovani* can provide protection against *L. mexicana* parasites by induction of a strong Th1 and suppressed Th2 cytokine response. These studies demonstrate the potential of live attenuated *L. donovani* parasites as pan *Leishmania* vaccines.

1020

CANDIDATE BIOMARKERS PREDICT PROGRESSION TO CHAGAS HEART DISEASE IN RURAL SOUTHERN BOLIVIA

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Nearly 30% of *Trypanosoma cruzi*-infected individuals eventually manifest Chagas cardiomyopathy (CC). A test for early detection of patients who will progress to CC is needed, as early treatment may improve prognosis. In this cross sectional study of a cohort in southern Bolivia, serum biomarkers and EKG changes were measured in 68 *T. cruzi*-positive individuals representing the various stages of *T. cruzi*-related heart disease and 17 individuals who were *T. cruzi*-negative and without evidence of cardiac disease. Chagas disease staging was assessed using the ACC/AHA heart failure classification system. Patients were classified as Stage A (*T. cruzi* positive with normal EKG/CXR), Stage B (*T. cruzi* positive with abnormal EKG but normal CXR), and Stage C (*T. cruzi* positive with abnormal EKG and cardiac structural changes). Markers Transforming Growth Factor (TGF) β1 and 2, Connective Tissue Growth Factor (CTGF), Matrix Metalloproteinase (MMP)2, MMP9, Tissue Inhibitor of Metalloproteinase (TIMP) 1, Brain Natriuretic Peptide (BNP), Mannose Binding Lectin (MBL), C-terminal Propeptide of Type I Procollagen (PICP), and N-terminal Procollagen III Propeptide (PIIINP) were measured using ELISA or Luminex. QRS scar score was calculated for each patient using EKG measurements. Individuals with Stage B had higher QRS Score ($p<0.001$), increased concentrations of MMP2 ($p=0.013$), TIMP1 ($p=0.003$), TGFβ1 ($p=0.023$), and TGFβ2 ($p<0.001$) as compared to those with Stage A. Multinomial logistic regression showed a strong association of increased concentrations of TIMP1 (RRR=1.94; $p=0.036$), TGFβ1 (RRR=2.05; $p=0.011$), and TGFβ2 (RRR=8.11; $p=0.037$) with a diagnosis of Stage B as compared to Stage A. A higher QRS Score (RRR=10.5; $p<0.001$) and increased concentrations of MMP2 (RRR=6.77; $p<0.001$) and BNP (RRR=2.75; $p=0.010$) were strongly associated with a Stage C diagnosis as compared to Stage A. Associations with markers of fibrosis and tissue remodeling (QRS Score, MMP2, and TIMP1) likely indicate the presence of advancing cardiac structural change. However, the elevated concentrations of immunoregulatory markers (TGFβ1 and TGFβ2) among those with Stage B disease may be clinically useful predictors of eventual development of CC. This information will be particularly important in the developing countries where Chagas disease is most prevalent and medical resources are limited.

1021

HIGH-THROUGHPUT SCREEN OF THE *TRYPANOSOMA BRUCEI* METHIONYL-TRNA SYNTHETASE ENZYME: INITIAL AND FOLLOW UP RESULTS

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Improved treatments for human African trypanosomiasis are urgently needed to replace existing drugs that have major liabilities including poor efficacy, high toxicity, parenteral administration, or high costs. In previous work, we have demonstrated by RNA interference that methionyl-tRNA synthetase (MetRS) is essential for growth of the etiologic agent, *Trypanosoma brucei.* In order to screen for new chemical compounds with inhibitory activity against the *T. brucei* MetRS we developed a high-throughput enzyme assay based on ATP-depletion and a bioluminescence readout. After miniaturization of the assay to the 1536-well format, the chemical library of 364,131 compounds at Scripps Florida was tested at a single point concentration of 11.9 μM. The assays had outstanding statistics with an average Z'-score of 0.90 +/- 0.03. Of the 1456 hits, 1370 were obtained and rescreened in triplicate demonstrating 1270 confirmed hits. Due to the high number of confirmed hits, an orthogonal assay was devised to try to eliminate potential false positive hits. This orthogonal assay quantified the production of AMP as measured by antibody binding using fluorescence polarization as the readout. The confirmation rate was 43.7%, yielding 599 hits. Based on bioinformatics analysis to select a broad diversity of chemical structures, a subset of 249 compounds was tested in the subsequent dose response assays against the MetRS. The luminescence assay confirmed 137 compounds with activity <10 μM and the fluorescence polarization assay confirmed 96 compounds. Representative compounds from twelve different structural clusters are currently being tested in follow up assays to assess specificity on the trypanosome over the human MetRS analogs, to measure growth inhibition on *T. brucei* cultures, and to explore the potential for hit-to-lead drug development.

1022

ROLE OF NK CELLS (CD56+ CD8+) IN THE PATHOGENESIS OF CUTANEOUS LEISHMANIASIS BY *LEISHMANIA BRAZILIENSIS*

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Patients with cutaneous leishmaniasis (CL) by *Leishmania braziliensis* has exacerbated T lymphocyte activation with production of cytokines such as IFN-γ and TNF and high levels of TNF-α associated with disease severity. Recently, attention has been described to the role of CD8 + T cells to deleterious inflammatory response observed in these patients. The role of cytotoxicity in defense mechanism in CL or pathogenesis in humans is not well understood. The main objective of this study is to determine the contribution of NK cells (CD56 +) and NK (CD56 + CD3- CD8+) to immunopathology observed in patients with LC. Using flow citometry and citotoxicity assay as methods to answer our questions, our data showed that the majority of the cells that are positive to grazime and perforin and they are NK cells. This population of NK express CD8 (CD8+ CD56+CD16+ CD4- iNKT-CD3-) and the frequency of these populations are increased in patients with CL compared to healthy subjects. Our preliminary data most interesting demonstrated that the majority of CD8+ T cells in lesions of patients with CL are actually NK cells (CD56 +) expressing CD8+. A sub-population characterized as being CD56+CD3+CD8+ when stimulated with soluble antigen of *Leishmania* (SLA) to verify the ability of these cells to produce IFN- γ had a frequency of 14.3%. In conclusion, this new population described here as CD56+CD8+ is important to citotoxicity in CL patients as a cell responsible to kill infected macrophages.

1023

A SELECTIVE AND IRREVERSIBLE BTK INHIBITOR, IBRUTINIB (PCI-32765) SUPPRESSES A TH2 RESPONSE AND INCREASES RESISTANCE TO CUTANEOUS *LEISHMANIA MAJOR* INFECTION

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Treatment for leishmaniasis, in addition to being expensive and highly toxic, is ineffective due to rising drug resistance. Hence, chemotherapies that modulate host immune response towards a leishmanicidal Th1 response are required. In this study we show that Ibrutinib (PCI-32765), a drug shown to be effective in Phase I/II lymphoma trials, is capable of modulating a Th1 dominated immune response during cutaneous leishmaniasis caused by *Leishmania major*. *L. major* infected BALB/c mice treated with Ibrutinib had similar lesion progression compared to vehicle treated *L. major* infected mice until six weeks of infection and subsequently developed smaller lesions from week-6 to week-9 post-infection. This is corroborated by significantly lower parasite burdens in the footpad lesions of week-9 Ibrutinib treated mice compared to footpad lesions from vehicle treated mice. Draining lymph node cells from week-9 Ibrutinib treated mice stimulated with *L. major* antigen produced significantly lower concentrations of IL-4, IL-10 and IL-13 compared to vehicle treated mice while ratios of IFN-γ:IL-4, IFN-γ:IL-10 and IFN-γ:IL-13 were significantly higher in Ibrutinib treated mice compared to vehicle treated mice. Serum IgG1 antibody titers at week-8 post-infection were significantly lower in Ibrutinib treated mice compared to vehicle treated mice while IgG2a titers were similar between the two groups. Thus, our studies indicate that Ibrutinib (PCI-32765) administration suppresses Th2 dominated immune response in *L. major* infection and enhances resistance. This drug requires exploration as a therapeutic agent against leishmaniasis.

1024

COMMUNITY BASED APPROACH TO COMMUNITIES WITH HIGH PREVALENCE OF *ANCYLOSTOMA DUODENALE, STRONGYLOIDES STERCORALIS* AND ANEMIA IN NORTHERN ARGENTINA

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Soil-transmitted helminth (STH) infections are the most prevalent neglected tropical diseases, causing anemia, malnutrition and negative consequences in growth and cognitive development of children. Among STH, *Strongyloides stercoralis* (Stst) deserves special consideration due to difficulties in diagnosis and the use of ivermectin for treatment. The aim of this study was to describe the prevalence and morbidity of STHs through a cross-sectional study and the usefulness and feasibility of a community based MDA program integrated to the public primary health care system in two Wichii aboriginal communities (Kilometro6 and Lapacho) in Tartagal, northern Argentina. A statistically representative group using the household as the unit of randomization was selected for surveillance. Single stool samples were analyzed with four methods: sedimentation, McMaster, Agar plate and Harada-Mori. Hemoglobin and antibodies titers against Stst using ELISA-NIE were measured. Single dose albendazole and ivermectin were used for treatment from March to December 2012. The study population included 2289 individuals, 157 had their stool analized. STH prevalence was 47% for hookworms, 13% for Stst and 2% for *Ascaris lumbricoides*. The cumulative prevalence of STH was 55%. Hookworms were all *Ancylostoma duodenale*. Stst seroprevalence was 50%. Anemia prevalence was 55%. Calculated coverage achieved 79%. Tartagal is an area of high prevalence of STH infections where preventive antihelminthic chemotherapy is indicated twice a year. Anemia is a severe public health problem due its high prevalence. The inclusion of ivermectin is justified by the prevalence of Stst, which was detected more frequently by serology. Pharmacovigilance revealed adequate safety of the drug regimen. Community treatment in a house-to-house approach is useful, particularly for risk groups such as preschool-aged children and women of reproductive age. The strategy of integrating the antihelminthic treatment to the primary health care system was successfull in achieving the acceptance of the comunity and high coverage.

1025

POTENTIAL IMPACT OF IMPROVED HELMINTH DIAGNOSTICS ON CONTROL AND ELIMINATION EFFORTS

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The successful implementation of MDA programs can decrease helminth burden and prevalence within communities. Given that WHO guidelines for treatment are based on microscopy screening to determine prevalence, it is important to consider the impact of other testing strategies in these populations. Traditional microscopy methods used to detect helminth infections have limited sensitivity compared with real-time multiplex PCR. However, this improved test performance may modify the ability to detect associations between helminth infection, risk factors, and clinical outcomes. This cross-sectional study was nested within an RCT conducted at 3 sites in Kenya. We performed microscopy and PCR for the stool detection and quantification of *Ascaria lumbricoides*, *Necator americanus*, *Ancylostoma duodenale*, *Srongloides stercoralis*, and *Schistosoma* species. We utilized regression to evaluate associations between potential risk factors or laboratory outcomes and infection as detected by either method. Of 307 adults surveyed, 61(19.9%) and 21(6.8%) were positive for one or more helminth species by PCR and microscopy, respectively ($p<0.001$). PCR-detected infections were associated with farming (RR 1.57, 95% CI: 1.02, 2.40), communal water source (RR 3.80, 95% CI: 1.01, 14.27), and lack of primary education (RR 1.54, 95% CI: 1.14, 2.33), whereas microscopy-detected infections were not associated with any risk factors under investigation. Microscopy-detected infections were associated with significantly lower hematocrit and hemoglobin (means of -3.56% and -0.77 g/dl) and a 48% higher risk of anemia (RR 1.48, 95% CI: 1.17, 1.88) compared to uninfected. Such associations were absent for PCR-detected infections unless infection intensity was considered. Infections diagnosed with either method were associated with increased risk of eosinophilia (PCR RR 2.42, 95% CI: 1.02, 5.76; microscopy RR 2.92, 95% CI: 1.29, 6.60). Differences in helminth diagnostic test performance alter associations between infection, risk factors, and clinical outcomes. As control efforts move to targeted populations and as global efforts shift from control to elimination, it is important to consider the potential implications of using improved diagnostic technologies for helminth infection. These findings suggest that while more sensitive diagnostics identify more infections overall, they may lead to the detection of a higher proportion of clinically less important infections.

1026

THE INTERRUPTION OF THE TRANSMISSION OF SOIL TRANSMITTED HELMINTHS BY PERIODIC MASS CHEMOTHERAPY OF SCHOOL AGED CHILDREN: TRANSMISSION THRESHOLDS AND THE SIGNIFICANCE OF NON-RANDOM CONTACT WITH INFECTIVE STAGES

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Previous analyses have highlighted the importance of understanding age-structured mixing to estimate the likely impact of school-based deworming programmes. However, there is no framework for identifying which aspects of the epidemiology of the parasite in the population and the implementation of the treatment program are most important in determining the bounce-back rate of infection following treatment and the opportunities for breaking the transmission cycle. We analyse an age-structured mixing model, in which school-aged children and adults contribute differently to the deposition and acquisition of infective material in the environment and school-age children are subject to periodic chemotherapy. Using a combination of newly derived analytical expressions and numerical simulation, we identify five key parameter groupings that determine the bounce back rate, the effective reproductive number and the point at which the transmission cycle is broken. We show how the bounce-back time (and therefore the critical treatment frequency) is influenced by the relative intensity of infection in the different age groups, the treatment coverage in school-aged children, the extent to which adults and children are exposed to each other's output of infective stages, the relationship between frequency of treatment and the timescale of the survival of infective stages in the environment. This work further illustrates the central importance of parasite sexual reproduction in the context of periodic chemotherapy. Previous work has shown that the effect of worm mate availability has a negligible effect on the undisturbed parasite population, but the pulsed nature of periodic treatment means that its impact can be considerable. Failure to include sexual reproduction in models can lead to significant underestimates in bounce back times and in efficacy of a given treatment regime. We discuss the implications of these findings for the design of treatment programs in different epidemiological settings.

1027

RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INFECTIONS DURING THE FIRST THREE YEARS OF LIFE IN THE RURAL TROPICS: ANALYSIS OF A BIRTH COHORT

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Soil-transmitted helminths (STH) infect more than 2 billion humans worldwide, causing significant morbidity in children. There are few data on epidemiology and risk factors for infection in pre-school children. To investigate risk factors for infection in early life, we analysed data collected prospectively in a birth cohort in tropical Ecuador. A total of 2,404 children were recruited at birth and followed up to 3 years of age with periodic collection of stool samples that were examined for parasites using modified Kato-Katz and formol-ether concentration techniques. A questionnaire to collect data on social, demographic, and environmental risk factors was administered to the child's parent at the time of birth. The outcome was defined as the detection of any STH infection during the child's first 3 years of life. STH infections were observed from 7 months of age and prevalence increased with age, with 35.3% of children having at least one STH infection to age 3 years. *Ascaris lumbricoides* was the most common STH infection (present in 28.5% of all infections) followed by *Trichuris trichiura* (16.8%), *Strongyloides stercoralis* (1.1%) and hookworm (0.7%). Independent risk factors for any STH infection in multivariate logistic regression were: Afro-Ecuadorian ethnicity (vs. Mestizo, adj. OR 1.93, 95% CI 1.54-2.42, P<0.001), low maternal educational level (illiterate vs. secondary, adj. OR 1.94 (95% CI 1.34-2.80, P<0.001), urban residence (vs. rural, adj. OR 1.44, 95% CI 1.15-1.81, P=0.001), household crowding (>3 vs. <3 people/sleeping room, adj. OR 1.40, 95% CI 1.13-1.73, P=0.002), and maternal STH infections (e.g. *T. trichiura*, adj. OR 1.68, 95% CI 1.34-2.10, P<0.001; *A. lumbricoides* infection intensity, >3rd tertile vs. uninfected, adj. OR 3.60, 95% CI 2.44-5.30, P<0.001). Our data show that over a third of children were infected with STH parasites during the first 3 years of life in an Ecuadorian birth cohort. Maternal geohelminth infections, and living in an urban environment in conditions of poverty were most strongly associated with the acquisition of STH infections.

1028

WATER, SANITATION AND HYGIENE-RELATED RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INFECTION IN URBAN SCHOOL- AND PRESCHOOL-AGED CHILDREN IN KIBERA, NAIROBI

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Urban slum dwellers have limited access to city services, including water, sanitation, and hygiene (WASH). WASH factors can affect risk for soil-transmitted helminth (STH) infections, which disproportionally affect school-aged (SAC) and preschool-aged children (PSAC), but further characterization is needed to identify potential interventions. Households containing a PSAC (6-59 months) or SAC (5-14 years) were randomly selected from those enrolled in CDC's International Emerging Infections Program, a population-based surveillance system in the Kibera slum in Nairobi, Kenya. Data collection included a household questionnaire and environmental assessment for WASH risk factors; stool specimens were tested for STH ova by the Kato-Katz method. WASH risk factors were tested for associations with STH infection using univariable and multivariable Poisson regression. STH prevalence among the 201 PSAC and 475 SAC meeting inclusion criteria was 40.8% and 40.0%, respectively. According to WHO/UNICEF water and sanitation ladder classifications, 3.1% of households reported piped water on premises versus 96.9% another improved water source, and 2.3% reported improved sanitation facilities versus 87.7% shared, 6.2% unimproved, and 2.3% open defecation. STH infection was significantly associated with household toilet located off premises (PR=1.33; p<0.05), while always treating water (PR=0.81; p=0.04), clean towel use during hand drying (PR=0.58; p<0.01), finished household floor material (PR=0.76; p=0.01), in-home electricity (PR=0.70; p<0.01), and 10 meter increases in household elevation (PR=0.89; p<0.01) were protective for STH infection. On multivariable analysis, STH infection was significantly associated with treating water usually versus always (aPR=1.5; p<0.01), while finished household floor material (aPR=0.76; p=0.02) and 10 meter elevation increases (aPR=0.90; p<0.01) were protective against infection. The Kibera population faces gaps in water availability and sanitation quality; several modifiable risk factors exist that could be suitable targets for STH control.

1029

RANDOMIZED PLACEBO-CONTROLLED TRIAL OF THE EFFICACY OF MEBENDAZOLE POLYMORPHS IN THE TREATMENT OF HOOKWORM INFECTIONS

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Mebendazole has three polymorphic forms, identified as A, B and C. It has been suggested that unlike polymorph C, A is ineffective in the treatment of hookworm and whipworm infections. A randomized double-blind, placebo-controlled trial was carried out to compare the efficacy of single dose 500 mg tablets of pure mebendazole Polymorph C with those containing a 1:1 mixture of Polymorphs A and C, for the treatment of hookworm infections. All eligible individuals living in 219 households were recruited after obtaining written, informed consent. A single fecal sample was obtained and examined the same day, using the Kato-Katz technique for intestinal nematode infections. Those who were found infected with hookworms were randomized to one of three treatment arms and requested to provide a second faecal sample 10 - 14 days after treatment. This was examined in the same manner as the first. A total of 892 individuals were recruited; 601 provided fecal samples; 214 were found positive for hookworm; 70, 74 and 70 individuals were randomized to treatment arms A (mixture of polymorphs A and C), B (pure polymorph C) and C (placebo) respectively. Follow-up samples were provided by 53, 48 and 49 persons respectively in each treatment arm. The cure rates in the three treatment arms were 28.3%, 18.8% and 16.3% respectively; they were not significantly different from one another. Comparison of fecal egg count reductions (FECR) in the 3 treatment arms (86.1%, 84.5% and -6.6% in Arms A, B and C respectively) showed that both mebendazole formulations performed significantly better than placebo, but there was no statistically significant difference between FECR with the two drug formulations. It is concluded that a single 500mg dose of mebendazole, either as Polymorph C alone, or as a mixture of Polymorphs A and C, has little efficacy in curing hookworm infections. However, both formulations were significantly better than placebo in reducing the intensity of infection, with no statistically significant difference between the two formulations.

1030

MUCOSAL IMMUNE RESPONSES DURING HELMINTH TREATMENT FOR INFLAMMATORY BOWEL DISEASES

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Helminth treatment for inflammatory bowel diseases has support from experiments in mouse models as well as clinical studies, but the mechanism of action is unclear. Based on a longitudinal analyses of an individual who self-infected with *Trichuris trichiura* to treat his symptoms of ulcerative *coli*tis, we hypothesize that enhancement of mucosal barrier function by TH2 immunity and IL-22 may improve conditions of ulcerative *coli*tis. In this patient, as well as additional other ulcerative *coli*tis patients, TH22 cells (IL-22+, IL-17-) were reduced in tissues with active inflammation and induced in tissues colonized by worms. We then conducted a trial where we treated macaques suffering from idiopathic chronic diarrhea with Trichuris trichiura, collecting biopsies before and after treatment for FACS analyses. A TH2 response was induced and 4 out of the 5 treated macaques improved their symptoms. We also found reduced bacterial attachment to the intestinal mucosa and identified changes to the composition of microbial communities attached to the intestinal mucosa post treatment. These findings suggest that helminth treatment may restore mucosal barrier functions, reducing overall bacterial attachment to the epithelium, and also altering the communities of attached bacteria. We are currently enrolling patients in a double-blinded placebo controlled trial to further investigate these mechanisms in human subjects, treated with *Trichuris suis* ova (TSO). The trial is designed to characterize mucosal responses to TSO treatment and to distinguish between responders and non-responders to TSO treatment.

1031

THE IMPORTANCE OF CONTEXT: HOW SOCIAL AND ENVIRONMENTAL FACTORS MODIFY THE EFFECT OF HEAVY RAINFALL ON DIARRHEA INCIDENCE

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The impact of heavy rainfall on water-borne diarrheal diseases is uncertain. This may be due to important biophysical and social factors that modify its effect. We aimed to estimate the effect of heavy rainfall on diarrhea incidence in northern coastal Ecuador, evaluating whether biophysical and social factors impact vulnerability to heavy rainfall events. Active surveillance for diarrhea was conducted weekly for 39 months in 19 villages. We defined heavy rainfall as one-day rainfall in a seven-day period exceeding the 90th percentile value within the study period. Mixed effects Poisson regression was used to test the hypothesis that prior rainfall, water and sanitation coverage and social cohesion modified the relationship between heavy rainfall and diarrhea incidence. We found prior rainfall and drinking water treatment modified the relationship between heavy rainfall and diarrhea. Heavy rainfall was associated with increased diarrhea incidence following 8-week periods of low rainfall (IRR 1.39, 95% CI 1.03, 1.87) and decreased diarrhea incidence following 8-week periods of high rainfall (IRR 0.74, 95% CI 0.59, 0.92). Drinking water treatment reduced the deleterious impacts of heavy rainfall following dry periods. When 67% percent of households reported drinking water treatment, the risk of diarrhea due to heavy rainfall was null (IRR 1.04, 95% CI 0.70, 1.54). Sanitation, hygiene and community social cohesion did not modify the relationship between heavy rainfall and diarrhea. Heavy rainfall appears to cause diarrhea through contamination of drinking water, and presents the greatest health risk following periods of low rainfall. Interventions to increase drinking water treatment may reduce climate vulnerability.

1032

MICROBIAL SOURCE TRACKING IN RURAL INDIA: UNDERSTANDING HUMAN AND ANIMAL CONTRIBUTIONS TO FECAL CONTAMINATION OF IMPROVED AND UNIMPROVED COMMUNITY WATER SOURCES, STORED DRINKING WATER AND HANDS

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To reduce the global diarrhea disease burden, the Millennium Development Goal (MDG) 7c aims to increase access to safe drinking water and basic sanitation. The MDG definition of improved drinking water, however, does not include assessment of microbial safety. Several studies in low-income countries have shown improved drinking water sources are contaminated with feces, and household stored drinking water can have higher levels of fecal contamination than source water due to contact with dirty hands. Identification of fecal pollution sources is necessary to better assess health risks and protect drinking water from high risk sources, especially in areas like rural India where animal and open human defecation occur together. Microbial source tracking (MST) using *Bacteroidales* genetic markers is an emerging approach to determine host contributions to fecal pollution.

The goals of this study were (1) to assess the level of human and livestock animal fecal contamination in improved (public and private tube wells for drinking), unimproved (open ponds) water sources, household stored drinking water and on hands in rural communities in India, using MST based on *Bacteroidales* host-associated markers, and (2) to examine the relative microbial safety of improved and unimproved water sources by simultaneously measuring selected pathogens. In 24 villages in Puri, India, 20-L samples of public (n = 43) and private (n = 41) tube wells and ponds (n = 38), 300-mL samples of household stored drinking water (n = 135), and hand rinses of mothers (n = 136) and children (n = 135) were collected in the monsoon season of 2012. After concentration of bacteria and viruses by filtration, fecal sources were determined using quantitative PCR assays validated in India to measure general, human- and bovine-associated markers. Diarrheal pathogens (rotavirus, adenovirus 40/41 and *Vibrio cholera*) in water sources were also tested via qPCR. An unfiltered portion was analyzed for fecal *coli*form. We present the MST and pathogen detection results and discuss the findings.

1033

A CLUSTER RANDOMIZED CONTROLLED EVALUATION OF THE HEALTH IMPACT OF A NOVEL ANTIMICROBIAL HAND TOWEL ON THE HEALTH OF CHILDREN UNDER TWO YEARS OLD IN RURAL COMMUNITIES IN NYANZA PROVINCE, KENYA

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Diarrheal diseases are responsible for 17.4% of deaths among children <1 year old globally. Although poor hygiene is an important contributing factor to this disease burden, promoting and sustaining effective handwashing is challenging. Handwashing with soap has been shown to prevent up to 53% of diarrheal, respiratory, and skin infections in young children in developing countries. To address this problem, an innovative technology was developed that consists of reusable hand towels with antimicrobial properties that are non-toxic and stable over time. We conducted a cluster-randomized, longitudinal study to evaluate the impact of the towels on child health. We selected enumeration areas (EAs) that were accessible year round, and randomized them into intervention and control groups. We conducted a baseline survey of mothers and then gave 4 towels to intervention households with handwashing education and handwashing education alone to control households. We made biweekly home visits for 1 year to ask mothers about diarrheal diseases, acute respiratory infections (ARIs), and skin infections in children <2 years old. We tested post-handwashing hand rinse samples of a random sample of 20% of mothers for *Escherichia coli*, an indicator of fecal contamination. At the end of the study, we tested a 50% sample of the towels for *E. coli* contamination. Of 449 enrolled households, 369 (82%) completed >75% of biweekly visits and were included in analysis. At baseline there were no significant differences between intervention and control households. During 8,555 total home visits, enumerators observed at least 3 towels in over 80% of intervention households. There were no statistically significant differences between intervention and control children in rates of diarrhea (1.45 vs 1.47, P=0.99), ARI (1.36 vs 1.50, P=0.57), or skin infections (1.86 vs 1.69, P=0.55) per hundred person-visits. There were no significant differences in post-handwashing hand contamination between intervention and comparison participants and 67% of towels were contaminated with *E. coli*. Antimicrobial hand towels became contaminated with use over time, and did not improve hand hygiene or prevent diarrhea, ARI, or skin infections.

1034

CHOLERA AT THE CROSSROADS: THE ASSOCIATION BETWEEN ENDEMIC CHOLERA AND NATIONAL ACCESS TO IMPROVED WATER SOURCES AND BASIC SANITATION

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Following major improvements to water and sanitation infrastructure, developed countries have been free of epidemic and endemic cholera for many decades. Most developing countries have made progress in increasing access to improved water and sanitation, but in many countries, cholera persists. The Millennium Development Goals include reducing by half the proportion of the world's population without sustainable access to safe drinking water and basic sanitation. We investigated the association between endemic cholera and national coverage with improved water and sanitation. We analyzed national-level WHO data on annual reported cases of cholera from 1991 to 2010 and estimates of urban and rural improved water and sanitation coverage from 1990, 1995, 2000 and 2005. We compared different definitions of endemic cholera, including the WHO definition of any indigenous cholera cases in ≥3 of 5 consecutive years and definitions using thresholds of annual counts of 10, 50, 100, 250, 500 and 1,000 cases. We performed logistic regression and generated Receiver Operating Characteristic (ROC) curves to assess the use of water and sanitation coverage levels as predictors of endemic cholera within succeeding 5-year periods. National estimates of access to both safe drinking water and basic sanitation were significant predictors of the occurrence of cholera at $p < 0.05$. Values under ROC curves ranged from 0.67 for water and 0.68 for sanitation using the WHO definition to 0.74 for water and 0.74 for sanitation using a definition of 250 cases per year in ≥3 of 5 consecutive years. An important limitation of the data is that many countries periodically fail to report cholera and almost never report counts of zero. This and other data limitations make it challenging to estimate a threshold value for national access to safe water or basic sanitation above which endemic cholera is no longer likely to be seen; however, enhanced definitions of endemic cholera result in improved sensitivity and specificity estimates.

1035

MODELING THE EFFECT OF WATER, SANITATION AND HYGIENE AND ORAL CHOLERA VACCINE IMPLEMENTATION IN HAITI

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In 2010, epidemic cholera was introduced to Haiti. Because resources are scarce, decision-makers need to understand the effect of different preventive interventions for ongoing transmission. We built a static model to estimate the potential number of cases averted by water and sanitation improvements (WASH) (i.e., latrines, point-of-use chlorination, and piped water), oral cholera vaccine (OCV), or a combination of both. We allowed for indirect effects and used non-linear relationships between effect and population coverage. We applied 1990-2010 cholera incidence data from Malawi to Haitian demographic data to estimate the potential annual incidence of endemic cholera for a 20-year period in Haiti. We modeled 16 scenarios: six WASH, six OCV, and four that combined WASH and OCV. Over the next two decades, scalable WASH interventions could avert from 57,949 to 78,567 cholera cases, OCV 38,569 to 77,636 cases, and interventions that combined WASH and OCV 71,586 to 88,974 cases. Rate of implementation is the most influential variable, and combined approaches maximized the effect.

1036

KNOWLEDGE, ATTITUDES AND PRACTICES REGARDING CHOLERA, SAFE WATER, SANITATION AND HYGIENE, AND IMMUNIZATIONS PRIOR TO AN ORAL CHOLERA VACCINE CAMPAIGN IN A REFUGEE CAMP - THAILAND, 2012

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Cholera is a major cause of morbidity in Mae La refugee camp along the Thailand-Myanmar border; 4 outbreaks occurred during 2005-2012. To complement ongoing safe water, sanitation and hygiene (WaSH) efforts, a preventive oral cholera vaccination (OCV) campaign was conducted in 2013 for all eligible camp residents. In December 2012, before the campaign, we interviewed one respondent in a cross-sectional sample of households (HH) using a standard questionnaire to assess knowledge, attitudes and practices (KAP) regarding cholera, WaSH, OCV and other immunizations, and anticipated OCV acceptability; the survey included HH water testing for residual chlorine and *Escherichia coli*, an indicator of fecal contamination.Among 271 HHs, median HH size was 5.6 persons and 52% had ≥1child aged <5 years. Respondents had lived in the camp for a median of 7.5 (range 1-31) years, median age was 39 (range 15-77) years, 76% were female, 79% were ethnic Karen, and 40% had never attended school. Although 81% of respondents had heard of cholera, only 52% identified watery diarrhea as a symptom. Only 25% respondents said that they treated drinking water to make it safer; of these, 66% boiled water and 9% treated water with chlorine. Soap use for handwashing was reported by 66% of respondents. Overall, 70% of respondents knew vaccines prevented diseases, and 85% of HHs had ≥1 member who ever received vaccines. Only 40% of respondents had heard of cholera vaccine, but 97% were willing to receive free OCV for themselves or their family members. Among stored HH drinking water samples tested, 8% had residual chlorine, and 39% were positive for *E. coli*. Despite frequent recent outbreaks, cholera awareness was low, and safe water and hygiene practices were infrequently used. Knowledge of OCV was low, but anticipated OCV acceptance was high. Preliminary results were used to emphasize cholera disease and WaSH messages during the OCV campaign. A post-campaign survey is planned to assess actual vaccine acceptance and impact of the OCV campaign on cholera knowledge and WaSH behaviors.

1037

CHOLERA VACCINATION CONTRIBUTES TO IMPROVED KNOWLEDGE REGARDING CHOLERA AND IMPROVED PRACTICE RELEVANT TO WATER-BORNE DISEASE IN RURAL HAITI

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The cholera epidemic in Haiti has been devastating partly due to underlying weak infrastructure and limited clean water and sanitation. A comprehensive approach to cholera control is crucial, yet influential international policy makers have argued that oral cholera vaccination (OCV) might reduce hand washing and hygiene among vaccine recipients. We aimed to assess the impact of an OCV program on knowledge and health practice in rural Haiti. We hypothesized that such a program, which included education on cholera and hygiene, would improve knowledge and behavior critical for cholera control. We administered surveys on knowledge and practice relevant to cholera and water-borne disease to every 10th household during a census prior to an OCV program in rural Haiti (N=811). We administered the same survey to 518 households randomly chosen from the same region 3 months after the OCV program and compared results using Statistical Analysis Systems (SAS 9.3). Post-vaccination, there was improved knowledge of cholera with significant increase in correct responses on cholera transmission (odds ratio [OR] 1.91; 95% confidence interval [CI] 1.52 - 2.40), preventive methods (OR 1.83; 95% CI 1.46 - 2.30), and means of treating water (OR 2.75; 95% CI 2.16 - 3.50). Relative to pre-vaccination, participants were more likely after OCV program to report always treating water compared to never, sometimes or often (OR 1.62; 95% CI 1.28 - 2.05) and washing hands with soap and water > 4 times a day (OR 1.30; 95% CI 1.03 - 1.64). In pre and post OCV surveys, knowledge of treating water as a cholera prevention measure was associated with practice of always treating water (OR 1.47; 95% CI 1.14 - 1.89). Post-vaccination, knowledge was associated with frequent hand washing (OR 2.47; 95% CI 1.35 - 4.51). Our study revealed that an OCV program in rural Haiti was associated with significant improvement in knowledge of cholera and practices related to water-borne disease. Cholera vaccine can be part of comprehensive cholera control and reinforce, not detract from, other control efforts in Haiti.

1038

CONCOMITANT HOUSEHOLD USE OF LYMPHATIC FILARIASIS INTERVENTIONS IN HAITI

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Early results from baseline and midpoint household surveys conducted for a multiyear prospective cohort study in the community of Ça Ira, Haiti explored the adoption of interventions against transmission of lymphatic filariasis (LF). Marketing of locally produced Bon Sel salt, co-fortified with iodine and diethylcarbamazine citrate (DEC), has been ongoing throughout the surrounding commune for over a year, and bed net distribution near the community began two years ago to supplement ongoing yearly mass drug administration (MDA) since 2000. The impact of marketing activities on consumption of salt as well as the interaction with bed net use had not previously been examined. Surveys were conducted in November 2011 (n=283) and February 2013 (n=232). Use of bed nets and Bon Sel rose from 48% to 62% and from 0 to 48% of households, respectively. Households reporting the use of at least one bed net were 2.20 (95% CI 1.28-3.79) times more likely to use Bon Sel than those who did not use a bed net in the house. Interviewees' opinions of the benefits of the product were significantly associated with the use of Bon Sel (P<0.001) and those who used Bon Sel were more likely not to wash their salt before consumption (P<0.001). The near-absence of salt washing among those who use Bon Sel supports the value of messaging to ensure the effective delivery of DEC. While those who did not use Bon Sel mostly had no opinion of the product, those who did consume it primarily identified it as being "good for your health". The increase in protective measures against LF occurring concurrently in the same households is a positive development, although households not using either protective intervention may indicate the presence of a persistent filarial reservoir, presenting a possible challenge for the national elimination program. Further study is needed to assess the association of these vulnerable households with non-participation in MDA activities.

1039

FOCAL DISTRIBUTION OF LYMPHATIC FILARIASIS AND OTHER HELMINTHIC INFECTIONS IN LIBERIA

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Pilot surveys were performed to identify villages suitable for studying the impact of mass drug administration (MDA) on helminthic infections in Liberia. Small cross-sectional surveys of adults assessed prevalence rates for lymphatic filariasis (LF) and other helminthic infections in 45 villages in Grand Bassa, Bong, Maryland and Lofa Counties (total population 1 million). National mapping for LF prior to our study was limited to two localities per county in most cases. Surveys of 564 adults in 11 villages in Grand Bassa County found a mean ICT rate of 3.2% with no microfilaria (Mf) carriers, while the mean ICT rate in 392 adults in 8 villages in Bong County was 2.6% with only 1 Mf carrier. However, the mean *Onchocerca volvulus* (Ov) nodule rate was 26% in the Bong county villages. ICT and Mf rates for 543 adults in 5 villages in Maryland County, Harper District were high (24% and 10%). Ov and schistosomiasis were absent in that area, but soil transmitted helminth (STH) infections were present in 50% of the screened individuals (primarily hookworm and *Ascaris*). Surveys of 1,092 adults in 21 villages in Foya district, Lofa county revealed mean ICT and Mf rates of 16% (range 0-26%) and 9% respectively, while the mean Ov nodule rate was 23% (range 0-47%). Subsequent studies showed high rates of hookworm infection (mean rate 61%) and *Schistosoma* mansoni (mean rate 88%) in Foya District villages. These pilot surveys have revealed distinct distribution patterns for different helminthic NTDs in Liberia. Lymphatic filariasis and onchocerciasis tend to be focally distributed by village or village cluster, while schistosomiasis rates are more uniform within districts. STH infections appear to be endemic throughout rural Liberia, with hookworm being the dominant species. These results provide a rationale for different mapping strategies for different helminthic NTDs in West Africa. It is difficult to justify the current practice of mapping focally distributed LF in just two localities within administrative units with populations that may exceed 200,000 (e.g., counties in Liberia). Two localities per district (with populations in the range of 50-200,000) may be sufficient for STH and schistosomiasis, but we advocate systematic, fine grained mapping for LF and Ov. This could be accomplished by mapping villages according to a grid system to provide data from one locality per 10,000 population.

1040

SEROPREVALENCE AND SPATIAL EPIDEMIOLOGY OF LYMPHATIC FILARIASIS IN AMERICAN SAMOA AFTER MASS DRUG ADMINISTRATION

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Significant progress has been made toward eliminating lymphatic filariasis (LF) from American Samoa. After seven rounds of mass drug administration (MDA) a population-based survey was conducted, and antigen prevalence by immunochromatographic test (ICT) had dropped from 16.5% (N=3018) in 1999 to 2.3% (N=1881) in 2007. In 2011, a WHO recommended Transmission Assessment Survey (TAS) was conducted among 1st and 2nd graders. Of 949 children tested by ICT, two positive cases were identified. Results from the TAS suggested that LF transmission had likely been interrupted and MDA no longer required. However, success of LF elimination programs depends on careful monitoring for potential resurgence of transmission after stopping MDA. Our study aims to provide information to direct future elimination activities by determining seroprevalence, assessing risk factors for infection, and identifying clusters at micro-spatial levels using household locations. Blood samples collected in 2010 from 807 adults in 55 villages were tested for LF antigen and antibody using Og4C3 and Bm14 ELISAs, respectively. Antigenemia was detected in 6 of 807 adults (0.7%, 95% CI 0.3-1.6%, 29-66 years old, 83% male). Bm14 antibodies were detected in 18.2% of samples (95% CI 15.6-21.1%, 18-77 years old, 71% male). Antibodies to Wb123 antigen will also be measured. Two apparent clusters of antigenemic individuals were identified using Kulldorff's spatial scan statistic, with relative risks of >18. Both antigen-positive children from the TAS attended the same school located in one of the clusters. Our study demonstrates the value of geospatial tools for identifying residual foci of infection, potentially allowing more targeted treatment and surveillance to reduce transmission and resurgence risk. Further research is required to identify reasons for clustering of infections (e.g. environmental, vectors, vegetation, or poor MDA uptake). If environmental risk factors are identified, predictive risk maps could be produced with geospatial modelling and used to direct future surveillance and interventions.

1041

PERFORMANCE COMPARISON OF THREE QUALITY OF LIFE INSTRUMENTS IN LYMPHATIC FILARIASIS PATIENTS

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Lymphatic filariasis (LF) is a parasitic infection that affects approximately 120 million people across 81 countries. Characterized by lymphedema and debilitating inflammatory episodes, LF significantly impacts health-related quality of life (HRQoL). As such, HRQoL is a frequently used outcome measure of LF intervention efficacy, despite the lack of a gold-standard LF HRQoL tool. To delineate the strengths and weaknesses of HRQoL tools in the LF population, the relative performances of three HRQoL tools were compared in LF subjects and an age- and sex-matched control group in Kerala, India. All subjects completed the World Health Organization Disability Assessment Schedule (WHODAS 2.0), a generic tool, the Dermatology Life Quality Index (DLQI), a skin-specific tool, the Lymphatic Filariasis QoL Questionnaire (LFSQQ), a disease-specific tool, and a demographic questionnaire. All three tools demonstrated decreased HRQoL in LF subjects as compared to the control group (WHODAS 2.0: 22 vs. 2, DLQI: 6.9 vs. 0, LFSQQ: 23 vs. 1, $p<0.001$). Discriminant validity was best assessed by the LFSQQ. The total WHODAS 2.0 score correlated strongly with the total DLQI score ($r= 0.75$, $p<0001$) and the total LFSQQ score ($r=0.91$, $p<0.001$). Global LFSQQ strongly correlated with total DLQI score ($r = 0.81$, $p<0.001$). Disease stage was not significantly associated with total QoL score of any tool but was weakly associated with the LFSQQ disease burden domain ($p=0.040$) and the DLQI symptoms and feelings domain ($p=0.045$). The DLQI yielded the lowest missing value rate (0%), and the WHODAS 2.0 domains displayed the best internal consistency (mean = 0.85; range = 0.76-0.91), although all tools demonstrated acceptable missing value rates and internal consistency values. Based on the high construct and discriminate validity and acceptable feasibility and internal consistency of the LFSQQ, we recommend use of the LFSQQ in LF HRQoL assessment.

1042

DEVELOPING AN INTEGRATED MICRO-MAPPING TOOL TO INFORM TREATMENT STRATEGIES FOR LYMPHATIC FILARIASIS ELIMINATION IN *LOA LOA* CO-ENDEMIC AREAS OF CENTRAL AND WEST AFRICA

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The wide distribution of *Loa loa* filariasis through Central and West Africa poses a major obstacle for national programmes scaling up mass drug administration (MDA) for the elimination lymphatic filariasis (LF). Standard drug treatment regimes (including ivermectin) cannot be used due to the risk of severe adverse events (SAEs), especially in medium (20-40%) to high loiasis (>40%) prevalence areas. Many countries lack high resolution LF mapping data and the extent of geographical overlap between these two filarial infections is not known. This study aimed to develop a simple method for potential LF- loiasis co-endemic areas to better classify *L. loa* risk, and to determine if integrated/LF micro-mapping and alternative treatment strategies are required to ensure safe and effective treatment. The geographical information system ArcGIS was used to import and overlap maps, and classify areas accordingly with colour coding. The recent loiasis prevalence map developed from RAPLOA surveys was used a base to define *L. loa* risk at sub-national level according to prevalence i) undefined (white) ii) low risk 40% (red). Based on these classifications it was recommended that sub-national areas with *L. loa* <20% required standard LF mapping and MDA treatment (i.e. green-proceed with standard strategy); areas with *L. loa* 20-40% required integrated LF-loiasis micro-mapping to determine extent of co-endemicity, and potentially alternative treatments (i.e. amber-caution and confirm best strategy); areas with *L. loa* >40% required only LF micro-mapping as high *L. loa* risk was already established and alternative treatments were essential (i.e. red-stop standard strategy). From this, a user-friendly colour coded map of sub-national areas in Central and West Africa was developed to help programmes quantify the number of high risk areas within each country, and areas that may benefit from alternative treatments including vector control, which will allow for more efficient use of financial and human resources.

1043

SEMI-QUANTITATIVE SCORING OF THE IMMUNOCHROMATOGRAPHIC CARD TEST RESULTS FOR CIRCULATING FILARIAL ANTIGENEMIA

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The value of scoring filarial antigen test (Binax Now Filariasis card test, ICT) results according to the relative intensities of the test and control lines was evaluated during a survey performed in a village in the Republic of Congo. 134 of 774 tests performed were clearly positive (17.3%), and 11 had questionable results (1.4%). *Wuchereria bancrofti* microfilariae (mf) were detected in night blood smears from 41 of 133 of those with ICT test scores of 1 or higher; mf were not detected in any of 11 slides from people with questionable ICT results. Cuzick's test showed a highly significant trend for higher microfilarial densities in groups with higher ICT scores ($p < 0.001$). Antigen test scores were also significantly correlated with mf counts in individuals. Filarial antigen levels provide an indication of adult worm infection intensity. Our results suggest that semi-quantitative ICT test scores may be useful for grading the intensity of filarial infections prior to treatment and for assessing the impact of treatment on adult *W. bancrofti* worms in individuals and in populations.

1044

EPIDEMIOLOGICAL, CLINICAL, AND LABORATORY EVALUATION OF ONCHOCERCIASIS IN AN AREA OF HIGH PREVALENCE - KITGUM AND LAMWO DISTRICTS, NORTHERN UGANDA 2012

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Onchocerciasis, a neglected parasitic disease, affects at least 37 million people globally. Efforts to eliminate this disease are based on mass drug administration of ivermectin. As part of a project aimed to evaluate tools for measuring the impact of elimination efforts, the authors performed convenience sampling in Kitgum and Lamwo districts in Uganda. The area had a baseline microfiladermia prevalence of 62% and nodule prevalence of 82%. The communities had received ivermectin for less than 5 years, with the last round distributed four months before the study. Risk factor and clinical data were collected on 500 individuals. Blood smears for *Loa* and *Mansonella*, immunochromatographic card tests for filariasis, and skin snips for onchocerciasis were evaluated on-site. Plasma, serum, blood smears, dried blood spots, and preserved skin snips were sent to CDC-Atlanta for further testing. The median age of participants was 32 years (range: 6-92 years); 304 (61%) were female. Onchocercal skin disease was present in 251 (50%) people. At least one nodule was present in 204 (41%) people, with a median of 2 nodules per person (range 1-16). Onchocercal eye disease was present in 40 (8%) people; 27 (5%) had microfilaria in the anterior chamber of the eye. The skin snip was positive for *Onchocerca volvulus* in 127 (25.4%) people. Among those with at least one positive snip, the mean load of microfilaria (MF), determined by averaging the number of MF in both snips, was 9.3 MF per snip (range: 0.5-105.5). On-site tests for other filaria were negative. The OV-16 antibody test was positive in 406 (84%) people. Nine (7%) of those with a positive skin snip were negative for OV-16 antibody. Their ages ranged from 6-60 years. All nine reported taking ivermectin last year. Three had skin nodules. Two had microfilaria in the anterior chamber of the eye. Additional laboratory testing is pending for onchocerciasis and other filarial infections. A better understanding of the performance of the OV-16 antibody test in the African context is needed to ensure proper usage by elimination programs.

1045

MICRO-STRATIFICATION OVERLAP MAPPING OF FILARIAL INFECTIONS IN SOUTH SUDAN

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The new Republic of South Sudan has a long history of filarial infections causing onchocerciasis, lymphatic filariasis (LF) and loiasis. Current detailed epidemiological data are scarce due to years of civil conflict, and the national neglected tropical disease (NTD) programme will need to conduct extensive baseline mapping in order to scale up efforts for control and elimination through mass drug administration (MDA). A concern to the expansion of the onchocerciasis and LF programmes is the risk of serious adverse events (SAEs) associated with the use of ivermectin in areas co-endemic with loiasis. To better define these risks, this study examined all past and present filarial data from published literature, and environmental factors associated with their geographical limits and overlapping distributions. Using micro-stratification overlap mapping (MOM) and

geographical information systems (GIS), all three filarial diseases were found to be co-endemic in the south western region, predominately in Western Equatoria State adjoining the border of neighbouring countries. Limited data were available for LF, however, historical onchocerciasis and loiasis distributions from the 1940s-80s significantly overlapped with those from mid-2000s, which were determined from large-scale REMO and RAPLOA surveys. Endemic blinding onchocerciasis caused by *Onchocerca volvulus* was associated with the extensive river system flowing into the Nile River basin, and overlapped with loiasis in the tropical forest - savanna mosaic areas at elevations 400-600 meters, where ferralsols soils were dominant and Chrysops silacea the main *Loa loa* vector. This 'co-endemic hot spot' close to international borders where these diseases also prevail, may pose a significant challenge for the NTD programme in South Sudan. Cross-border coordination and further micro-mapping will be required to define the extent of overlap, identify key risk factors and determine the most appropriate safe and effective treatment strategy, which may include alternative drug regimes and/or integrated vector management.

1046

A CASE STUDY OF RISK FACTORS FOR LYMPHATIC FILARIASIS IN A VILLAGE IN THE REPUBLIC OF CONGO

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A study was conducted to identify risk factors associated with *Wuchereria bancrofti* infection in the Republic of Congo. The study was performed in Seke Pembe, a village located approximately 250 km of Brazzaville. Among the 774 subjects aged ≥5 years, 145 (18.7%) had positive filarial antigen tests (ICT card test), and 41 (5.3%) had microfilariae (mf) in night blood smears. The following factors were investigated: sex, age, use of bednets, use of pit latrines, source of water and hunting/fishing activities. Data were analyzed by mixed multivariate logistic regression models. The prevalence of infection (either by ICT or by mf) increased with age up to 16-20 years and remained stable thereafter. The analysis using filarial antigenemia as the dependent variable demonstrated an increased risk for males (odds ratio=2.0, 95% confidence interval [1.3-3.0], p<0.001) and for people who hunt or fish (OR=1.7 [1.1-2.7], p=0.013), and a protective effect of private pit latrines (OR=0.6 [0.4-0.9], p=0.009). Analyses restricted to males showed that those hunting or fishing by night had an increased risk for antigenemia (OR=2.0 [1.1-3.8], p=0.028), and hunting was also a risk factor for females (OR=2.5 [1.1-5.7], p=0.036). Bednet usage was protective for the latter (OR=0.3 [0.1-0.9], p=0.03). There was a strong household effect for females (intraclass correlation coefficient [ICC]: 31%, p=0.0168), but not for males. In the analysis using mf as the dependent variable, males had a higher risk for mf positivity (OR=5.4 [2.1-13.4], p<0.001), private latrines had a protective effect (OR=0.4 [0.1-0.9], p=0.036) and a marked household effect was found (ICC=48.8%, p<0.001). Our results suggest that the effects of these major risk factors are already evident before 20 years of age. Age, sex, and occupation-dependent exposure to mosquitoes seem to be important risk factors for infection with *W. bancrofti*. It appears that males often acquire infections in high transmission areas outside of the village whereas females are infected in areas with lower transmission inside or near the village.

1047

HEALTH IMPACT ANALYSIS OF LYMPHATIC FILARIASIS ELIMINATION VS. ERADICATION SCENARIOS

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The neglected tropical disease lymphatic filariasis has been targeted for eradication by 2020. While great strides have been made to reach this target based on annual rounds of mass drug administration (MDA), a number of endemic countries, many with high levels of prevalence, or co-endemicity with onchocerciasis or loaisis, are behind schedule or are targeting only a small proportion of their at-risk population with their MDA programmes. We present an analysis that compares the health impact expressed as disability adjusted life years lost on a year to year basis over the next half century when current levels of rollout and coverage are maintained (leading to elimination in certain areas, but not affecting disease burden in other areas) to a scenario where a scaling-up to effective levels of coverage occurs everywhere and is maintained until elimination is achieved (eradication). Prevalence of symptomatic disease was calculated using a deterministic model of lymphatic filariasis transmission, Epifil, with parameter values corresponding to an Indian (transmission by *Culex* mosquitoes) and African (transmission by *Anopheles* mosquitoes) setting, while the health burden for 62 countries was calculated using country-specific demographic parameters. Countries were categorized by transmission archetype, depending on the relevant vector genus, initial prevalence, and treatment. A sensitivity analysis accounting for rates of disease progression, demography, and discounting, is presented. Due to the long-lasting nature of symptomatic disease there is a considerable lag between an elimination effort and the accrual of DALYs averted; the consequences of this pattern for cost-effectiveness of interventions will be discussed.

1048

DEVELOPING ALTERNATIVE DRUG DELIVERY STRATEGIES FOR LYMPHATIC FILARIASIS ELIMINATION IN URBAN AREAS IN GHANA

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Lymphatic Filariasis (LF) is a significant health problem in developing countries. It causes of permanent disability and undermines the social and economic welfare of affected populations. Mass drug administration (MDA) with ivermectin and albendazole is the strategy for elimination of lymphatic filariasis. MDA in urban areas is a major challenge for elimination programmes. The capital of Ghana, Accra has recorded low MDA coverage over the last 4 years and this study investigated the reasons for this low coverage recorded in urban communities. The objective was to identify the opportunities and barriers for implementing MDA in urban settings in order to develop appropriate strategies for urban MDA. This cross-sectional exploratory and interventional study was carried out in 2011 and 2012. It employed both qualitative and quantitative methods involving household surveys with community members, in depth interviews with health providers and community stakeholders and a transect walk in sampled communities. Urban communities are not uniform and need different but specific strategies to reach its diverse communities and residents. Urban populations are educated and require knowledgeable distributors to respond to pertinent questions such as side effects. All segments are not reached due to inadequate number of community drug distributors (CDDs) deployed while others are engaged to work in communities they are not familiar with. Inadequate social mobilization to create awareness on the importance of participating and complying with the treatment is important. Poor CDD remuneration remains a major challenge. The role of health workers in facilitating MDA in urban areas is central. Recognizing and responding to the needs of varied and sophisticated communities to increase their participation and compliance

in MDA is important together with enhancing social mobilization and improved remuneration of CDDs. Good leadership and supervision are essential to urban MDA activities.

1049

THE IMPACT OF IVERMECTIN ON ONCHOCERCIASIS AND ITS BURDEN OF MORBIDITY AND MORTALITY IN SAVANNAH SETTINGS OF AFRICA

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Mass drug administration with ivermectin has successfully contributed to control onchocerciasis as a public health problem, and in some foci of Latin America and Africa, elimination of the infection reservoir appears to have been achieved with the use of ivermectin alone. However, as control policy shifts from morbidity reduction to elimination at a pan-African scale, it becomes necessary to evaluate the long-term impact of ivermectin both on parasite populations and disease burden. We developed a mathematical model of the dynamics of onchocercal disease by linking previously established associations between infection and morbidity/mortality to output from an onchocerciasis transmission dynamics model. We assessed the long-term impact of mass drug administration with ivermectin on the prevalence and intensity of onchocercal infection and its associated disease burden in savannah areas of Africa, and explored how this impact is influenced by different epidemiological and programmatic scenarios. We assumed that ivermectin efficacy remained unchanged during the programme. Long-term annual ivermectin treatment is highly effective at reducing both morbidity and mortality associated with onchocerciasis, with projected results being relatively insensitive to treatment coverage and compliance. By contrast, overall impact on infection prevalence and intensity is highly dependent on pre-control endemicity, therapeutic coverage and systematic non-compliance, the latter warranting further investigation particularly as programmes advance towards elimination. Our results also indicate that excess host mortality associated with onchocerciasis is a substantial component of its overall disease burden, hitherto underestimated.

1050

SCENARIOS FOR LYMPHATIC FILARIASIS ERADICATION AND THE ASSOCIATED IMPACT ON NUMBER OF TREATMENTS NEEDED

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Lymphatic filariasis (LF), a neglected tropical disease causing chronic morbidity, affects an estimated 120 million people. In 2000 the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was established with the goal of eliminating LF as a public health problem within 20 years. While great gains have been made towards controlling LF, elimination by 2020 is unlikely to be achieved in many areas. Scenarios that are more likely to achieve eradication of lymphatic filariasis than the current GPELF strategy are presented that take into account the adaptations necessary to accommodate the disparity between different LF endemic environments. For each scenario, the total number of treatments for Mass Drug Administration (MDA), either administered as albendazole and ivermectin or albendazole and diethylcarbamazine (DEC), is estimated. The analysis utilizes the most current projections of the at-risk population for LF, as listed in the WHO's Preventive Chemotherapy databank, as well as the results of deterministic modeling to predict the number of MDA rounds required to achieve local elimination in given transmission settings. The model takes into consideration different transmission archetypes, including the three different LF species (W. *bancrofti*, B. malayi and B. timori), the primary vectors (*Anopheles*, *Culex*, *Aedes*, Mansoni), and the different MDA protocols (albendazole with DEC or ivermectin). Varying levels of scale up and MDA coverage are considered, with optimistic and pessimistic scenarios developed. The number of treatments required to maintain control or to achieve eradication is compared against each other, as well as against the most recent GPELF's projections and planning documents in order to determine whether a higher level of commitment and resources than currently allocated is necessary to reach eradication.

1051

URBAN TRANSMISSION OF LYMPHATIC FILARIASIS AND OTHER NEGLECTED TROPICAL DISEASES IN URBAN SETTINGS IN TANZANIA

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Over the last decades there has been considerable increase in urbanization in Africa. Estimates show that by 2030 over half of the population will be living in urban areas. The rapid growth of towns and cities, combined limited economic opportunities, often results in expansion of informal settlements and slums, with favourable conditions for proliferation of vectors and for transmission of many of the neglected tropical diseases (NTDs). This study aims to investigate the epidemiology of lymphatic filariasis (LF) and other NTDs, in two urban settings in Tanzania, Dar es Salaam (metropolis) and Tanga (a smaller city). The study thus addresses issues of high relevance for the control of these infections in urban settings. Pupils from different urban zones of Dar es Salaam and Tanga were screened for infection with LF, urinary schistosomiasis and STH. Nearby communities were examined for LF infection and disease. The human examinations were accompanied by surveys for vector snails and mosquitoes, by questionnaire surveys on hygienic and socio-economic conditions, and by characterization of the environments with regard to water, drainage, sewerage and garbage disposal facilities. The occurrence of LF and other NTDs in the schools and communities (and occurrence of vector mosquitoes and snails) were analyzed in relation to environmental and socio-economic risk factors in the examined urban settings. The usefulness of screening pupils for antibodies to Bm14 for the identification of risk areas for transmission of LF was also assessed. Selected aspects of the findings will be presented. The results from the studies will be used for recommending appropriate surveillance and control measures for LF and other NTDs in urban settings.

1052

EVALUATION OF THE TOURNIQUET TEST FOR THE DIAGNOSIS OF DENGUE INFECTION

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Dengue fever (DF) represent a major public health problem worldwide and cause a spectrum of illness ranging from the self- limiting dengue fever to more severe, life-threatening forms of the disease termed dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Brazilian Ministry of Health defines as dengue with complications (DCC) every severe case that does not fit the WHO criteria for DHF. The tourniquet test (TT) has been recommended as a tool to differentiate dengue from other acute febrile illness. The aim of our study was to evaluate the usefulness of the TT among suspected dengue patients. We recruited 30 patients and questionnaire was used to collect clinical and laboratory data. The clinical and nonspecific laboratory data were collected from all patients. The TT was considered positive if 20 or more petechiae were identified. In this study 30 patients with suspected dengue were analyzed, 60% were female and 40% were male. Regarding the clinical form of the disease, 21 (70%) had DF, 5 (16.6%) had DCC and 4 (13.3%) had

DHF. A TT was positive in 33.33% patients, 50% were negative and 16.67% were not performed. With the analysis of blood count, average hemoglobin found was 13.03g/dL, the mean hematocrit was 38.7%, and the average number of leukocytes was 4965.20/mm3. The average of lymphocytes 39.7% and platelets was 113,903mm3. Of DF patients, 62% had hemorrhagic manifestation, 47.6% had leukopenia, 47.6% had thrombocytopenia. Eighty percent of patients had thrombocytopenia and also had hemorrhagic manifestation. All cases of DHF showed warning signs, including persistent vomiting, severe abdominal pain, drowsiness/irritability, fainting and oliguria. In conclusion, DF was the most prevalent classification in this study and all cases of DHF showed warning sign. The TT has been recommended as a tool to differentiate dengue from other acute febrile illness but in this study this method showed small positivity.

1053

EFFECT OF FORMULATION RATIOS AND DOSING SCHEDULES ON THE SAFETY AND IMMUNOGENICITY OF A RECOMBINANT LIVE ATTENUATED TETRAVALENT DENGUE VACCINE (DENVAX) IN HEALTHY ADULT VOLUNTEERS

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We have been developing a tetravalent, live attenuated dengue vaccine (DENVax) consisting of a molecularly characterized, attenuated DENV-2 strain and three chimeras in which the prM and E genes of the attenuated DENV-2 were substituted with those of DENV- 1, -3 or -4 viruses. Tetravalent formulations of DENVax have been shown to be safe and effective in Phase 1 clinical studies. To further optimize the vaccine, a phase 1b clinical trial was performed to evaluate the safety and immunogenicity of various formulations and administration schedules in healthy, flavivirus negative adults. Two formulations were evaluated which varied in the amount of DENVax-4 virus and included subcutaneous administration of one or two doses on Day 0 with or without one or two doses on Day 90. A total of 115 subjects were evaluated in 5 different groups. To date there have been no serious adverse events. Two subjects discontinued; one for possible arthritis and one for Grade 3 arthralgia/myalgia for one day. The most common systemic adverse events included headache (39%), fatigue (35%), muscloskeletal disorders (35%), and myalgia (27%). The only Grade 3 lab abnormalities were elevated creatine phosphokinase (CPK) values in three subjects following exercise. The vaccine was well-tolerated with mostly mild and transient local reactions. In addition, DENVax induced significant neutralizing antibody responses to all four dengue viruses after one or two administrations. This study highlights the safety and immunogenicity of the different tetravalent DENVax formulations and schedules of administration.

1054

EPITOPE MAPPING OF ENHANCING ANTIBODIES PRODUCED BY THE HUMAN IMMUNE RESPONSE AFTER PRIMARY DENGUE VIRUS INFECTIONS

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Dengue virus (DENV) is a mosquito-borne flavivirus that causes over 390 million new infections globally each year and has become a major public health concern. About 500,000 DENV-infected individuals develop the more severe forms of dengue. DENV exists as four serotypes, named DENV1 through 4. Following a primary infection, individuals produce a mixture of type-specific and cross-reactive antibodies. Pre-existing immunity is sufficient to protect against re-infection with the same serotype, but may increase disease severity during a secondary infection with one of the other three DENV serotypes. A leading theory to explain the higher frequency of severe disease is the antibody-dependent enhancement (ADE) theory, where pre-existing DENV-specific antibodies are thought to bind viral particles and facilitate infection of host cells through Fcγ receptors. Due to the complexity of the humoral immune response, the DENV enhancing antibodies within human polyclonal sera after natural infections has not been well characterized. In the present study, antibodies in DENV-immune human sera were fractionated using recombinant viral proteins, and the role of specific antibody (Ab) populations in DENV enhancement was investigated. Depleted DENV-immune human sera were tested for the ability to enhance DENV in cell culture and in the AG129 mouse model of infection and disease. Enhancement studies with depleted human sera demonstrate that a fraction of enhancing Abs is targeted to the DENV recombinant envelope (rE) protein. The rE protein also contains the fusion loop, to which previous studies have mapped cross-reactive, weakly neutralizing Abs. Many enhancing human monoclonal Abs have also been mapped to the pre-membrane (prM) protein. Competition ADE studies with prM-binding Fab fragments show that prM-binding Abs in human immune sera contribute to ADE of heterotypic serotypes. Further studies are currently being conducted with recombinant pr protein (i.e. the cleaved peptide of prM) to further confirm the role of prM-binding Abs in ADE of heterotypic virus infection.

1055

A STUDY OF DENGUE RISK THROUGH THE LENS OF COMPLEX ADAPTIVE SYSTEMS (CAS) THEORY

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Social and ecological changes have caused tremendous variation in the human experience within cities of the Global South, including migration rates, fecundity, social interactions and bio-physical conditions of the residence. All four of these processes have been implicated in the emergence of dengue, but how they interact in the transmission process is unknown, partly because they occur over much larger temporal and/or geographic scales than those at which the mosquito *Aedes aegypti* transmits dengue between people. I present a study of dengue risk through through the lens of complex adaptive systems (CAS) theory and argue that a CAS approach to dengue research can fundamentally transform dengue control in endemic cities and increase the time between epidemics. CAS can be defined as a heterogeneous group of individual agents whose interactions evolve over time based on the outcome of those interactions. A useful way to study CAS dynamics is to frame the system as the interaction between short and long term processes which, in turn, affect the interaction between the system's internal dynamics and external processes. This approach lends nicely to the study of dengue transmission, which is influenced by the interaction between the fast and fine-scale processes of *A. aegypti* life history and slow and multi-scale processes of human life history. I review previous work demonstrating the potential explosiveness of dengue transmission across a group of houses during an inter-epidemic period in Colombia and model its relationship to these larger-scale urbanization processes, using longitudinal data on the mosquito dynamics collected from the same neighborhood and city-wide epidemiological surveillance data. Both the dynamics and socio-ecological drivers of local dengue transmission qualitatively change over time due to interactions between viral introduction from the rest of the city and the human life history processes within the blocks. Due to the spatial aggregation of *A. aegypti* recruitment, vector control efforts will be most effective in areas with increased migration and fecundity, but are ineffective and potentially counterproductive when social interactions facilitate high rates of viral introduction. These findings show that

local dengue transmission has typical CAS properties and highlight the opportunity to use socio-demographic conditions to pattern control strategies in space and time in heterogeneous cities.

1056

REVERSE TRANSCRIPTION-RECOMBINASE POLYMERASE AMPLIFICATION ASSAY FOR RAPID DETECTION OF DENGUE VIRUS

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Dengue is one of the most important arboviral diseases of human in tropical and subtropical regions of the world. A proper patient management and control of disease spread can be achieved with early and rapid detection of dengue virus (DENV) in patients during the viremic phase. Nucleic acid amplification and detection techniques for dengue diagnosis are available but its application in rural areas of the world where resources can be limited has been challenged due to the need for costly equipment. The recombinase polymerase amplification (RPA) assay is a novel isothermal technology for nucleic amplification and detection without the need for expensive equipment. Here, we evaluated a one-step single-tube reverse transcription-RPA (RT-RPA) assay developed by TwistDx Ltd. for the detection of all four DENV serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). The RT-RPA reagent mixture including the primer set was lyophilized and vacuum-packed to improve the storage stability and to simplify the operating procedure. The diagnostic coverage, sensitivity and specificity of the RT-RPA were evaluated using DENV RNA extracted from DENV infected cell culture supernatant. The RT-RPA assay detected the whole panel of 11 reference DENV strains circulating mainly in the major dengue endemic regions. The RT-RPA assay showed high sensitivity, comparable to the quantitative RT-polymerase chain reaction (qRT-PCR) as it detected up to 10 copies of the viral RNA. The RT-RPA can be completed within 15 minutes. There was no cross reactivity of the RT-RPA with other closely related arboviruses including Japanese encephalitis virus, Chikungunya virus and Sindbis virus. The RT-RPA is a suitable laboratory method for routine dengue diagnosis in rural clinics and field situation where resources are limited.

1057

CHALLENGES FACING DENGUE SURVEILLANCE AND CONTROL - DRAWING ON CASE STUDIES FROM BRAZIL AND INDIA AND THE NEED FOR INNOVATIVE APPROACHES

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The reemergence of dengue has become a major global health issue, responsible for 50-100 million infections worldwide. The changing epidemiology of dengue in recent years has been striking and encompasses the emergence or reemergence of different strains in Central and South America, the explosion of cases in India, and increasing sporadic cases in non-endemic countries due to travel and migration. Challenges for surveillance include the lack of uniformity in case definitions, laboratory capacity and different public health practices between countries. The political repercussions of acknowledging epidemics or true case load are also important considerations. This presentation focuses on lessons learned from cases in Brazil and India, particularly regarding the need for political will and cooperation. Examples include the reluctance to acknowledge the emergence of DENV-4 in Brazil and phylogenetic analyses to identify the origin of the strains. The unwillingness to document the increasing incidence in dengue infections that India is currently facing is also a pressing and timely issue. Greater attention must be paid to surveillance strategies and changing risk factors including climate change and rural-urban shifts. As a result of globalization, our world is increasingly interconnected and there is a call for novel collaborative approaches such as international networks and partnerships to share lab resources or data. Examples of innovation include redesigning vector control strategies to utilize chemoattractant and mechanical mosquito traps, which have been shown to be cost-effective particularly in high-risk areas. There is great hope that efforts to produce an effective vaccine will prove successful but this will undoubtedly raise many questions regarding the need to shift disease control priorities. However key control strategies will remain; such as understanding behavioral risk factors, improving environmental sanitation, raising public heath awareness, producing realistic estimates of the costs involved, developing and maintaining political will and international cooperation. The advent of event based surveillance systems such as ProMED or HealthMap has revolutionized our ability to potentially respond to epidemics. Other innovative surveillance strategies, such as designing early warning systems to provide a lead-time for authorities to adapt preventive measures, also hold great promise.

1058

SEROPREVALENCE OF DENGUE IMMUNITY AMONG MULTIPLE NONHUMAN PRIMATES IN SENEGAL

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Sylvatic dengue 2 viruses have been isolated from multiple primate species in Senegal. However, the importance of particular species of nonhuman primates to ongoing transmission of dengue in these settings is unknown. Here, we report age-stratified seroprevalence of DENV-2 antibody among African green monkeys (*Chlorocebus sabaeus*), patas monkeys (*Erythrocebus patas*), and Guinea baboons (*Papio papio*) captured over three years (2010-2012) in Kedegou, Senegal. Serum samples were collected from 219 African green monkeys, 78 patas, and 440 baboons that were captured, sedated and then released. The age of each primate was determined using an algorithm that used dental measurements, weight and other anthropometric data. We used this information to estimate the force or hazard of infection of DENV-2 for each species in each season. We find that DENV-2 hazards are high in all species with forces of infection of 0.69 (0.57, 1) for african green monkeys, $\lambda = 0.23$ (0.14, 0.38) for patas monkeys and $\lambda = 0.53$ (0.47, 0.6) for baboons. We also examine heterogeneities by troop and season. Our data suggest sustained, intense transmission of DENV-2 in each of these nonhuman primate species.

1059

EVALUATION OF SAMPLE INTEGRITY USING COMMERCIALLY-AVAILABLE RNA-STABILIZING BLOOD COLLECTION TUBES

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Degradation of RNA during specimen transport from collection site to diagnostic facility is a major problem affecting accurate diagnosis of RNA virus infection; speed and cold chain resources are not always available to maintain sample integrity. In this study, we used dengue as a model RNA virus to compare three commercial-off-the-shelf (COTS) RNA-stabilizing blood collection tubes for their ability to stabilize viral RNA in whole blood. We tested these products at conditions simulating low-viremia

samples (102 pfu/mL DENV1 spiked into whole blood) subjected to a loss of cold chain in a tropical environment (37 °C for 0, 8, 16, or 24 hours then restored to room temperature), reproducing possible obstacles in field transport. Using qRT-PCR we found that Biomatrica RNAgard tubes, Tempus Blood RNA tubes, and PAXgene Blood RNA tubes were all able to effectively stabilize dengue viral RNA even when present at low concentrations for up to one month; however, compatibility of these products with qRT-PCR was dependent on the RNA extraction method utilized. Investigations into the use of these products with downstream serological applications demonstrate that they may not be compatible with IgG ELISAs due to high background OD.

1060

ANTIBODY RESPONSE AGAINST SALIVA OF *AEDES AEGYPTI* AND *AE. ALBOPICTUS* IN INDIGENOUS DENGUE PATIENTS IN TAIWAN

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Dengue is one of the most predominant mosquito-borne infectious diseases. Salivary proteins of mosquitoes containing anti-haemostatic, anti-inflammatory, and immunomodulatory agents that contribute to the success of blood meal. Some of these molecules might involve in the transmission and establishment of pathogens in the hosts, disease progression or epidemic intensity. In Taiwan, *Aedes albopictus* is widespread whereas *Ae. aegypti* is prevalent in the southern counties where most dengue epidemics located. However, due to the frequent transportation on the island, the roles of these two capable dengue vectors played in the dengue epidemiology were yet to be elucidated. In this study, we aimed to investigate human antibody responses against *Ae. aegypti* and *Ae. albopictus* for a better understanding of the roles and functions of mosquito salivary proteins. The results of ELISA showed that dengue patients from southern Taiwan exhibited stronger antibody responses against *Ae. aegypti*, which supported the idea that the mosquito was the major vector responsible for serious dengue outbreaks in southern Taiwan. On the contrary, the readings of antibodies against *Ae. albopictus* were higher in dengue patients of northern Taiwan where only *Ae. albopictus* could be found. Nevertheless, cross-reactions were observed in most sera. Species-specific antigens by applying the pre-absorption procedures before Western blot revealed less than 10 immunogens in the mosquito salivary proteins. After pre-treating sera with salivary gland extracts of *Ae. aegypti*, bands with molecular weights about 44 kD, 35 kD, and 33 kD were absorbed against *Ae. aegypti* while bands with molecular weights about 290 kD, 68 kD, 44 kD, 36 kD, 35 kD, 29 kD, and 15kD were remained against *Ae. albopictus*. The potential applications of these immunogenic salivary proteins that served as biomarkers for exposure to dengue-infected mosquitoes will be discussed.

1061

ADJUSTMENT FACTOR FOR THE REPORTED NUMBER OF DENGUE CASES FROM A PROSPECTIVE COHORT IN PUNTA PRINCESA, CEBU CITY, PHILIPPINES

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Dengue illness is one of the eight pervasive infectious diseases in the Philippines. This study aims to provide an empirical estimate of the adjustment factor around dengue reporting based on a cohort study in Punta Princesa, Cebu City, Philippines. Monthly incidence rates (cases/1,000 population) of symptomatic dengue cases were compared between passive surveillance data from the Cebu City Health Department and active surveillance data from a prospective cohort in Punta Princesa. Case definition of symptomatic dengue was based on the Philippine Integrated Disease Surveillance and Response Manual in accordance to revised international health regulations of the World Health Organization. The surveillance phase of the cohort study included weekly SMS, phone call or home visit to check on the health status of 1,000 enrolled volunteers. Volunteers with acute febrile episodes had acute blood samples tested for serotype-specific dengue virus by hemi-nested reverse transcription-polymerase chain reaction (nested RT-PCR) and acute/convalescent blood samples tested by dengue IgM/IgG EIA in March 2012-March 2013. Results for March-October 2012 samples showed an average reporting rate of 13.28% of the confirmed symptomatic dengue cases in Punta Princesa by Cebu City Health Department, leading to an adjustment factor of 7.2 for converting reported cases into estimated actual cases. This is comparable to 7.0 overall expansion (adjustment) factor from a regression equation derived by Undurraga et al from reporting rates across Southeast Asia. This study is relevant in providing an empirical and critical estimate of the expansion factor. It is the first such study in the country, and makes the Philippines one of only 6 countries in Southeast Asia with such an empirical estimate. Such expansion factors are critical is determining a country's economic and disease burden of dengue fever.

1062

DENGUE IN MEXICO: USE OF MULTIPLE DATA SOURCES TO ESTIMATE ECONOMIC AND DISEASE BURDEN

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Despite its control in the 1970s, dengue incidence and severity have increased in Mexico since 1979, currently affecting 23 of 31 states, including the Texas-Mexico border area. Dengue is a reportable disease; Mexico's Ministry of Health (MoH) has promulgated protocols for laboratory confirmation and compiles and disseminates surveillance data. However, as in most countries, surveillance systems are not designed to report every case, so empirical adjustments are needed to extrapolate the overall number of symptomatic dengue episodes. Accurate information about the burden of dengue is needed to set health policy priorities and decisions about disease-control technologies. We merged multiple data sources to estimate (i) total episodes, (ii) costs per episode, (iii) surveillance and vector control costs, and (iv) disease burden using disability-adjusted life-years (DALYs) for 2010-2011. We estimated total episodes of dengue by multiplying reported cases (41,333 episodes in 2010, and 20,548 in 2011) by expansion factors derived from a cohort study in Morelos (2011-2012), and previous empirical estimates. We derived costs per episode from sequential interviews of 36 patients in four major hospitals in Quintana Roo, Morelos, and Tabasco; macro-costing data from two major public hospitals; MoH surveillance data; and previous literature. Indirect costs were based on productivity losses by age. Vector control and surveillance costs were estimated based on MoH data. Preliminary estimates suggest Mexico has about 345,000 dengue episodes (95%confidence interval [CI]: 204,000-434,000) and 135 dengue deaths annually on average. These amount to US$240 million (95%CI: 138-328

million) in illness costs plus US$89 million in federal vector control efforts, totalling US$329 million or US$3.02 per capita (95%CI: 2.08-3.82), and 16,000 DALYs per million population (95%CI: 8,800-22,700). With this study, Mexico joins Panama, Puerto Rico, and Thailand as the only countries or areas worldwide with comprehensive (illness and preventive) empirical estimates of the cost of dengue. These results reaffirm that exploring approaches to control dengue further would be economically valuable.

1063

ECONOMIC COST OF DENGUE VECTOR CONTROL IN MALAYSIA

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Dengue is endemic in Malaysia, with 46,171 cases reported in 2010 from its population of 28.4 million. The economic cost of dengue is an important policy tool to guide research around new and existing technologies. Vector control against *Aedes* mosquitoes is implemented by the Ministry of Health (MOH), district health offices (DHOs) and local councils (LCs). A previous study derived the cost of dengue illness in Malaysia, but we are not aware of any empirical study of the cost of vector control activities. We estimated public sector dengue control costs as part of a study evaluating the Malaysian dengue vector control program. We analysed costs from the vector control unit of the MOH plus these activities in 7 representative, statistically sampled districts from Malaysia's 160 districts. In each unit selected, both capital and recurrent expenditures were included and both DHOs and LCs were studied in sampled districts. Cost data for the year of 2010 were collected using data collection forms through detailed on-site interviews. Inputs were recorded in a matrix by line item and function. Line items consist of personnel, administrative and storage buildings, vehicles, fumigation equipment, pesticides, personal protective equipment and out-sourcing (fumigation services subcontracted to private companies). Functions consist of inspection, entomological surveillance, fumigation, larvaciding and health education. The analysis extrapolated costs from the sampled districts to the country, estimating both cost per capita and cost per registered dengue case. Costs were converted from Ringgit Malaysia (RM) to US dollars at the 2010 rate (RM3.2=US$1). Preliminary data give mean (± standard deviation) cost per district of aggregate vector control costs ($1.52 ± $0.79 in million), cost per dengue case ($702 ± $138), and cost per capita ($1.70 ± $0.21). Breakdowns by line item showed that human resources was the largest category (58% of costs) followed by pesticides (14%). Breakdowns by function found that fumigation activities had the highest share (36%) followed by inspection of premises (20%), with entomological surveillance as the least costly function (11%). Malaysia is joining the handful of countries worldwide with a comprehensive estimate of dengue costs, combining both illness and prevention. These tabulations are informing the public and stakeholders in at all levels about the economic impact of this disease.

1064

IMPROVING UNDERSTANDING AND SURVEILLANCE OF HUMAN PLAGUE IN NORTHERN UGANDA THROUGH MODELING AND COLLABORATION WITH TRADITIONAL HEALERS

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Plague is a highly virulent zoonotic disease that can cause bubonic, septicemic, or pneumonic illness in humans. If recognized early, plague can be treated successfully with inexpensive antimicrobials. Without treatment, more than 50% of bubonic cases are fatal. Although plague occurs worldwide, the overwhelming burden is in rural, impoverished areas of sub-Saharan Africa. During 2004-2009, 97% of the over 20,000 plague cases reported to the World Health Organization were reported from countries in Africa. In northwest Uganda, up to 400 cases are reported annually, with a case fatality rate of nearly 30%. Efforts at the National Center for Atmospheric Research in collaboration with the U.S. Centers for Disease Control and Prevention and the Uganda Ministry of Health have employed meteorological fields and epidemiological data to simulate the spatial and temporal variability of plague in the West Nile region of northwestern Uganda, in order to understand the drivers of the disease, and to identify under-served areas with elevated plague risk. Building on this work, in an effort to reduce the burden of illness in an area with little access to western health care, a training module has been developed and successfully implemented. This module is designed to expand surveillance by bringing together traditional healers and clinical practitioners and is aimed at reducing incidence of plague in rural Uganda. Results from this ongoing, interdisciplinary study will be presented.

1065

ICOPA XIII: THE CHALLENGE OF ORGANIZING FOR THE FIRST TIME AN INTERNATIONAL CONGRESS OF PARASITOLOGY IN A LATIN AMERICAN COUNTRY

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Mexico City was selected as the site for the next International Congress of Parasitology (ICOPA) to be held in August 10-15, 2014. Previous meetings have taken place in Melbourne 2010, Glasgow 2006, Vancouver 2002, Hokkaido 1998, Izmir 1994, Paris 1990, Brisbane 1986, Toronto 1982, Warsaw 1978, Munich 1974, Washington DC 1970 and Rome 1964. To assure that an inclusive and high quality program is organized, the local scientific committee has asked renowned parasitologists from all over the world and Presidents of National Societies of Parasitology to suggest symposia. The recommended speakers are being selected based on the number of publications in the last five years and the h index using the Scopus database. Up to now we have received and analyzed around 400 speakers distributed in 100 symposia, which gather most parasites related to human diseases as well as veterinary and fish parasites. Areas of research are also very diverse and include immunology, epidemiology, cell biology, molecular biology, ecology and many others. Preliminary data indicate that 54% of potential speakers live in America, 31% in Europe, 8% in Asia, 6% in Oceania and 1% in Africa. Their average number of publications in the last 5 years is 43 and the h index is 25, the latter measures productivity and impact of the published work (i.e. an h=20 indicates that 20 of all articles of an author have been cited at least 20 times). A further aim is to have a green paperless congress, thus we are

making a big effort to organize electronic poster presentations in which each participant will have 30 min for discussion of his work presented as "poster-oral" in a defined program. At the 62nd ASTMH Annual Meeting an up-dated scientific program, including plenary conferences, symposia and poster-oral presentations will be presented.

1066

PATTERNS OF GROWTH FAILURE IN INFANTS IN A RURAL DISTRICT OF PAKISTAN

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Malnutrition is major underlying factor in childhood morbidity and mortality in developing countries. Prevalence of chronic malnutrition is high in Pakistan, with a recent study showing 50% of the children being stunted at 18 months of age in a rural district. There is little data regarding patterns of growth failure in children in Pakistan. This pattern is important to study so the highest risk age for growth failure can be identified and appropriate interventions can be planned accordingly. We are conducting a longitudinal observational study of childhood growth monitoring in Matiari, a rural district in Sindh province of Pakistan. We have enrolled 817 infants between 0-1 months of age and are recording their weight and height at monthly intervals. Morbidity data regarding history of upper respiratory tract infections, diarrhea and fever is also being recorded on fortnightly basis. We found that the average weight for age z-score (WAZ) at 1 month was -1.68 for girls and - 1.84 for boys, which decreased further to -1.82 in girls and -2.06 in boys at 6 months of age. At 12 months the mean WAZ increased to -1.75 for girls and -1.99 for boys. Average height for age z score (HAZ) at 1 month was -1.64 in girls and -1.75 in boys, which decreased further to -1.76 in girls and -2.07 in boys at 6 months of age. At 12 months the mean HAZ decreased further -2.3 for girls and -2.65 for boys. This detailed analysis of growth faltering pattern in children will help identify the ages when the children are most at risk for growth failure so appropriate interventions can be planned accordingly.

1067

DENGUE VACCINE INITIATIVE PROJECT: A MULTI-COUNTRY STUDY OF THE ECONOMIC BURDEN OF DENGUE FEVER IN VIETNAM, THAILAND AND COLOMBIA

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Estimation of economic burden of dengue illness is helpful in understanding economic benefits and cost-effectiveness of dengue control strategies and improves evidence base for decision-making. The Dengue Vaccine Initiative is conducting cost of illness (COI) studies on dengue at facility-based fever surveillance sites in Vietnam, Thailand, and Colombia. The COI study estimates the direct and indirect costs associated with lab-confirmed inpatient and outpatient dengue cases in societal perspective. A series of two to three interviews are conducted using a survey instrument to collect the private direct out-of-pocket costs for services, medicines, diagnostics and transport; and indirect cost due to productivity loss among patients and their care takers. At the same time, the study teams collects cost information regarding the treatment provided at collaborating facilities. The total COI per case will be calculated from the sum of out-of-pocket payments, indirect costs, and the facility treatment cost net of any patient co-payments. From October 2011 to March 2013, a total 152 cases in Vietnam, 29 cases in Thailand and 9 cases in Colombia were enrolled in the study. Of the total 143 Vietnamese cases analyzed, 55 were hospitalized while the rest 88 were treated on outpatient basis. The average directs cost was $94 per inpatient and $40 per outpatient. Of the total direct inpatient costs, 30% was spent before visiting the hospital, 24% during the visit and 46% after the hospital visit. The respective direct outpatient costs were 25%, 8% and 67% before, during and after visit. The wage loss in inpatients and their care givers was 12 days and 8 days while outpatients and their care givers had lost wages for 8 days 2 days respectively. In the next step, total COI per case for Vietnam will be estimated and will continue data collection in Thailand and Colombia. The preliminary cost of illness result from Vietnam indicates that dengue illness is associated with substantial out of pocket costs and indirect productivity loss.

1068

INFERRING THE EFFECTS OF COINFECTION ON HUMAN HEALTH: COMBINING RESULTS FROM A SYSTEMATIC REVIEW AND A THEORETICAL MODEL

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Simultaneous infection by multiple parasite and pathogen species is commonplace in humans. Coinfection by parasitic worms alone affects more than a billion people. On top of this are viruses, bacteria, protozoa, and fungal pathogens. How this coinfection affects human health in terms of morbidity and mortality is poorly understood, and how best to treat coinfected individuals is an open question. To address these issues we combined two different, but complementary approaches. First, we systematically reviewed recent publications on coinfection in humans to (i) synthesise the reported impacts of coinfection in humans on morbidity and pathogen abundance, and (ii) characterize any reported interactions among parasites, the parts of the body they infect or consume, and any immune responses they elicit. Most publications reported that coinfections tend to raise pathogen load within coinfected hosts, and exert a greater health burden relative to the effects of single infections. Furthermore, pairs of coinfecting parasites had more potential to interact indirectly via their host, rather than directly, and these indirect effects were more often mediated by shared sites of infection than by shared immune responses. Second, we carried out simulations of a novel theoretical model of two interacting coendemic helminth species in a human population where one species was treated. We found a range of possible outcomes of targeted treatment on the non-target species but, in general, cotreatment of parasites that are natural enemies (those that interact antagonistically with each other) is advisable, whereas species-specific treatments of 'friendly' parasites (those that interact synergistically) confers greater benefits to host health than may currently be appreciated. Taken together, our results suggest that coinfection often, but not always, has deleterious effects on human health, and that knowing and understanding how coinfecting parasites interact may inform optimal treatment strategies for coinfected individuals.

1069

CLINICIAN TRAINING IN SELECT AGENT OUTBREAK RESPONSE IS POTENTIALLY SUSTAINABLE IN RESOURCE LIMITED, EAST AFRICAN COUNTRIES

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The United States Army Medical Research Institute of Infectious Diseases (USAMRIID) works closely with The United States Defense Threat Reduction Agency - Cooperative Biological Engagement Program (DTRA-CBEP) and the Ugandan NGO, Makerere University Walter Reed Project, to develop clinician training on early recognition, management and reporting of outbreaks of select agents (e.g., Ebola, anthrax) that is host nation sustainable in the East African Region. Kenyans and Ugandan leaders are (1) effectively engaged in designing and implementing training that improves clinician outbreak response, (2) have the skills and human resources to conduct and sustain the training, (3) committed to devoting time to training without remuneration. Key leaders from Kenyan and Ugandan government ministries, universities and non-governmental organizations met with USAMRIID during a workshop hosted over 3 days in Kampala, Uganda to: Provide constructive criticism of training material presented by USAMRIID, Delineate format and content of the curriculum, Identify and refine specific, measurable, attainable, relevant and timely goals, Develop criteria for selecting trainers-of-trainers, Define the target audience / end-user of the training, Define collaborator roles (consultation, accreditation, implementation, etc.), Establish a timeline for implementing the training, and Begin logistical planning for the first "training of trainers" iteration. Participants filled out Target Asset Maps covering 9 areas of necessary training related activity to inventory their roles and contributions to the training. All of the key objectives were addressed and consensus for a plan and timeline was achieved. Various participants had prior experience and willing to perform in all 9 areas of necessary training related activity; they were committed to working a range of 2-10 hours a week without remuneration. Kenyans and Ugandans consider the training necessary enough to develop their own plan and commit skills and time without remuneration. The training should proceed and funding procured.

1070

EFFECT OF DISTANCE FROM HOUSEHOLD ON UTILIZATION OF CAMPAIGN DISTRIBUTION POSTS DURING AN INTEGRATED CHILD HEALTH CAMPAIGN IN MADAGASCAR

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An important concern in planning public health programs is ensuring access to health services and interventions for the target population. This is especially challenging in developing countries with limited resources. Physical distance to health facilities has been associated with accessibility and utilization of routine health services; however, little information is available to assess access to supplemental health services such as child health campaigns. In this study we assessed the effect of distance between household and distribution post on access to interventions during an integrated campaign conducted in October 2007 in Madagascar that delivered measles vaccine, mebendazole, and vitamin A to children 6-59 months of age in 111 districts and long-lasting insecticidal nets (LLINs) to children aged <5 years in 59 districts. Six months after the campaign, a nationwide, community-based survey was performed using a two-stage cluster sampling design. Personal digital assistants equipped with global positioning systems (GPS) were used to collect coordinates of all households in a cluster, select a simple random sample, and enter interview data. In addition, the GPS location of the vaccine/LLIN distribution post was collected for each cluster. Over 51,100 households were mapped and a household member was interviewed in 4302 households from 180 clusters. Most households reported the location of the distribution post to be in their village (76.6% LLIN districts, 62.4% non-LLIN districts). The majority of households reported time to the distribution post to be <30 minutes (80.7% LLIN, 65.2% non-LLIN) or 30 minutes to 1 hour (12.0% LLIN, 22.8% non-LLIN). No significant difference was found in the median distance to a distribution post among children who attended the campaign (326 meters LLIN, 476 m non-LLIN) compared to those who did not (444 m LLIN, 498 m non-LLIN; LLIN p=0.22, non-LLIN p=0.54). In general, these results indicate the distribution posts were well distributed and accessible. GPS is a valuable tool for the evaluation of program activities.

1071

USING PAPER ANALYTICAL DEVICES AND A CELL PHONE TO MEASURE IODINE LEVELS IN URINE

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Urinary iodide (UI) levels are used to assess populations of their risk for iodine deficiency disorders. Most labs measure UI using the Sandell-Kolthoff reaction, in which iodide catalyzes the reaction between arsenic(III) and cerium(IV). The decrease in yellow Ce(IV) is monitored with a spectrophotometer. Prior to analysis, urine samples must be boiled in acidic ammonium persulfate to remove interferents, which requires access to laboratory facilities. A field-friendly paper analytical device (PAD) has been created that could revolutionize the way monitoring agencies test UI. The device requires no power, trained personnel, or instrumentation other than a cell phone. To use the device, the user places 2 drops of urine onto the reaction area and times how long it takes the indicator spot to turn from blue to red. The PAD measures physiologically relevant iodide concentrations at the parts per billion (ppb) level and allows detection of 25 ppb I within 6 minutes. We will focus on several innovations in the implementation of the Sandell-Kolthoff reaction on paper millifluidic devices: 1) We will report a redox indicator that gives much stronger colorimetric results suitable for interpretation by eye or by image analysis programs; 2) We will compare the accuracy and reproducibility of results gathered by video capture with results gathered from a single timed photograph; 3) We will report whether on-paper purification and other analytical strategies can successfully compensate for interferents in urine, eliminating the acidic oxidation step; and 4) Using the principles of life-cycle analysis during the design of the PAD, we incorporated reagents that generate iron oxide nanoparticles in order to entrap the toxic arsenic compounds used in the Sandell-Kolthoff reaction. We will report ICP measurements showing that on-PAD remediation successfully reduces leaching of arsenic from the PAD by over 95%, and that these PADs no longer qualify as toxic waste by the EPA's TCLP assay.

1072

DENGUE VACCINE INITIATIVE PROJECT: A MULTI-COUNTRY STUDY OF THE HOUSEHOLD WILLINGNESS TO PAY FOR DENGUE VACCINES

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As dengue vaccines approach licensure, potential demand for introduction should be examined for policymakers to make evidence-based decision on vaccine introduction. The DVI is conducting multi-country studies on the private willingness to pay (WTP) for dengue vaccines to assess population level perspective of the importance of the vaccine. To estimate household demand and WTP for hypothetical dengue vaccines, we administered a study questionnaire to 400 households in three countries: Nha Trang in Vietnam, Ratchaburi province in Thailand, and Medellin in Colombia. The hypothetical vaccine scenarios were composed of 70/95% effectiveness protective for 10/30 years, respectively. Five pre-assigned prices were determined to reflect local situations. Respondents were asked how many vaccines and for whom they would purchase within their household at randomly pre-assigned prices. The average household size is 4.81 in Vietnam (*N*=400), 5.1 in Thailand (*N*=400), and 4.92 in Colombia (*N*=190). About 28% of the households in Vietnam,and Thailand, as well as 9% of the households in Colombia, have experienced dengue fever. About 90% of respondents have understood the vaccine scenario presented. The raw data show that the average household demand for the hypothetical dengue vaccine is 2.7 at the lowest price and 0.8 at the highest price in Vietnam, 3.9 and 2 in Thailand, and 4.48 and 0.5 in Colombia. These clearly show that there is a higher demand for dengue vaccine at a lower price. Either a Poisson or negative binomial regression model will be chosen as an economic count model depending upon over-dispersion issues in the data from each site. The count model will be used to calculate average WTP and to estimate the average number of vaccines demanded as a function of the price, efficacy, perceptions of dengue severity, as well as household socio-economic characteristics. Non-parametric models will be measured for the youngest child. Both parametric and non-parametric estimates of average WTP provide information regarding the private benefits of vaccination, and enable comparison of household characteristics which may affect purchasing decisions across the countries. Preliminary results indicate that the people are willing to pay for dengue vaccine and the private demand is more likely to be influenced by price than by vaccine effectiveness. More data will be presented at the conference.

1073

A COST-EFFECTIVENESS ANALYSIS OF HUMAN AND PIG VACCINATION STRATEGIES TO REDUCE THE BURDEN OF JAPANESE ENCEPHALITIS IN BANGLADESH

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Japanese encephalitis virus (JEV) is a zoonotic, mosquito-borne *Flavivirus* that circulates among Ardeid birds and can be amplified by pigs. Despite the availability of safe and effective vaccines, Japanese encephalitis (JE) causes an estimated 67,900 human cases each year worldwide and has recently been recognized as an important cause of acute encephalitis and long-term neurological sequelae in Bangladesh. To inform decision-making for future vaccine introduction, we evaluated the cost-effectiveness of human vaccination in three regions of Bangladesh where population-based incidence estimates are available: Rajshahi, Khulna, and Chittagong. We compared the status quo (no vaccination) to a feasible intervention: incorporation of the SA-14-14 2 vaccine into the Expanded Programme on Immunization. The analysis was conducted from a societal perspective and incorporates both direct and indirect costs measured in 2010 US dollars. We also assessed the cost-effectiveness of annual vaccination of domestic pigs to reduce human cases in Rajshahi, where detailed data on pig populations are available. We conducted a 2-way uncertainty analysis of the pig vaccination model because the proportional contribution of pigs to human cases and costs associated with implementation of pig vaccination are unknown. Interventions were considered very cost-effective (VCE) below a threshold equivalent to the *per capita* GDP ($1700) per disability adjusted life year (DALY) averted and cost effective (CE) below a threshold of 3X the *per capita* GDP ($5100). Routine childhood vaccination is expected to be VCE, with a cost per DALY averted of $144 in Rajshahi, $264 in Khulna, and $763 in Chittagong. Probabilistic sensitivity analysis indicates that childhood vaccination has >97% probability of being VCE in Rajshahi and Khulna and 77% probability of being VCE in Chittagong. Vaccination of domestic pigs in Rajshahi would be cost-effective relative to both the status quo and childhood vaccination for all scenarios where pigs are responsible for >30% of human cases and the cost per pig vaccinated is <$10. Our findings suggest that including JEV vaccine into the EPI program would be a cost-effective approach to reduce death and disability from JE in Bangladesh, particularly in Rajshahi and Khulna. The contribution of pigs to human JE in Bangladesh should be quantified to determine if pig vaccination could also be a cost-effective public health strategy.

1074

DENGUE IN INDIA: THE MADURAI CASE STUDY AND NATIONAL IMPLICATIONS

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Dengue is a notifiable disease in India since 1996, with an annual average of 20,018 laboratory confirmed cases reported from 2006-2012. However,

the true magnitude of dengue burden is poorly understood due to scarcity of diagnostic tests, limited scope of the surveillance system, and other constraints. An accurate understanding of the dengue burden will help estimate the economic cost of this illness, assist policy makers in evaluating the benefit and effectiveness of various prevention and control technologies, and develop a combination of strategies to control dengue. A case study to estimate an adjustment factor to project the true number of dengue cases was conducted in Madurai District in the state of Tamil Nadu. A descriptive inventory of all health facilities or laboratories treating or testing dengue patients in the district was developed. The laboratories were classified by type of dengue test performed and hospitals were stratified by bed capacity. Numbers of suspected and confirmed dengue cases for the years 2009 through 2011 were obtained from the public laboratory for all public ambulatory facilities and a stratified sample of 12 of the 250 hospitals. Dengue cases from hospitals were extrapolated based on the ratio of total beds to beds in sample facilities by stratum. Projected dengue cases from the case study were compared with the officially reported numbers from the district surveillance unit (DSU) to the state level. The state-level estimate was then adjusted for the share of cases tallied at the national level. The DSU reported an annual average of 126 confirmed dengue cases, compared to a projected annual average of 3,324 cases from this case study. On an annual average, the DSU captured 86 (37%) of the 231 confirmed dengue cases treated in public hospitals and 48 (2%) of the 2,167 confirmed dengue cases treated in private hospitals, but none of the 905 laboratory-confirmed ambulatory cases, and none of the 1.3 dengue deaths. For laboratory confirmed dengue cases, the reporting rate at the state level (126/3324) was 3.73% and the expansion factor (3,324/126) was 26.4, and the national expansion factor was 47.1. Applying the national expansion factor to the reported cases suggests that India had an annual average of 943,000 confirmed dengue cases. This case study provides the first empirical estimate of an expansion factor for India, and the country experiences almost a million confirmed dengue cases annually.

1075

DEVELOPMENT OF A SERVICE MODEL FOR MOBILE DATA COLLECTION, CLOUD-BASED REPORTING AND CENTRALIZED DATA MANAGEMENT TO SUPPORT MULTIPLE NTD PROGRAMS

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The rapid expansion of mobile networks globally, coupled with the increasing functionality and decreasing cost of mobile equipment allows global health projects to increasingly utilize mobile and web-based technology tools in their work. The development and support for a simple-to-use mobile data collection system running on Android devices, coupled with a cloud-based reporting and data management system will make data collection faster, cheaper, and more accessible to programs wanting to utilize electronic data collection. While many tools exist to collect electronic data, most systems require considerable time and resources to move into production. By building a shared system as a service model we are able to provide servers, personnel, mobile equipment, and ongoing support at low cost to programs. Methods: Using the open source tool, Open Data Kit, we developed a modified survey application to streamline data entry, plus a web-based reporting website to expand online reporting and management of collected data. Over the past two years the LINKS System has been used in over twenty-five projects, surveys have been conducted by nine lead organizations (the largest to date being the Global Trachoma Mapping Project) and have resulted in the successful collection of millions of geo-referenced, easily-accessible data points. In conclusion, using a cloud based centralized system for smartphone data upload and web-based reporting services, we have developed a system that can be deployed on a large scale, across multiple countries, at low cost.

1076

MEASURING IMPACT OF SUPPLY CHAIN INTERVENTIONS USING A DIFFERENCE-IN-DIFFERENCES APPROACH

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SC4CCM is a learning project that tests simple, affordable solutions to address unique supply chain challenges faced by community health workers to generate knowledge that can be put into broader practice for supply chain operations, financing, and advocacy. The project developed a Theory of Change (TOC) to serve as a technical framework to guide the monitoring of change, the demonstration of success, and serves as the umbrella framework for country-specific TOCs. The interventions proposed to strengthen community supply chains are based on analyzing the relative strength of the system performance elements in the TOC and their preconditions based on data gathered at baseline in both countries. In Ethiopia, a group training approach was employed to improve supply chain management knowledge, skills, and tools among health workers. In Malawi, two interventions were implemented to improve community level product availability. One used a team approach to create a customer service oriented supply chain, while the other addressed transportation efficiency. To measure the impact of these interventions on product availability over time - a difference-in-differences (DiD) approach was used to subtract the effect of changes in supply chain outcomes in the comparison areas from changes in the intervention areas during the study period. Intervention and comparison groups were matched using relevant characteristics at baseline. Each DiD model was designed to take into account components from country-specific causal pathways in the TOC. The results for both Ethiopia and Malawi interventions did not find significant effects of the interventions on product availability, likely due to overlapping effects of factors introduced after the design of the interventions, affecting product availability in both groups. In the absence of significant DiD results, the TOC provided the project with a robust way to measure the true impact of the interventions and led the project to look deeper into causal pathway indicators to draw out the lessons from the interventions.

1077

IMPROVING COMMUNITY LEVEL SUPPLY CHAIN PERFORMANCE USING TEAM-LED, DATA DRIVEN SOLUTIONS IN MALAWI AND RWANDA

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In Malawi and Rwanda, community health workers (CHWs) treat children under five for pneumonia, diarrhea and malaria in their villages. Baseline assessments in 2010 identified gaps in supply chain knowledge, skills, procedures, information and decision making all of which contributed to low responsiveness to stockouts by CHWs and their managers. To address these barriers, SC4CCM partnered with 3 districts in each country to design and implement a series of interventions to first improve knowledge, skills and logistics data visibility, and then to promote a team-led approach to identifying solutions to close the supply chain performance gaps. While each country specific intervention was named and customized to reflect the local context, the team-based approaches had four key elements in common: 1) Teams consisting of CHWs and district and health center (HC) staff; 2) Joint identification of problems, development of plans that include targets for improvement; 3) Mechanisms for using data for performance monitoring and problem solving techniques for developing solutions; and 4) Use of recognition or rewards for motivation. Mixed-method evaluation surveys were conducted in early 2013, consisting of quantitative data collection from 10 districts in each country. In Malawi, 76% of CHWs

and 96% HC staff participated in meetings and over 80% of CHWs and HC staff knew their performance plans and targets,. Key performance monitoring indicators improved as well; reporting rates went from 43% at baseline to consistently above 90% for the six months prior to the survey, and reporting completeness was 90%. In Rwanda, monitoring data results showed 83% of HCs had full team member participation in meetings, 92% of HCs had team members who used the correct tool to identity SC performance problems, and 100% of HCs had teams that completed performance graphs after the last meeting. We concluded, therefore, that providing multi-level teams with a structure and process to identify, monitor and address problems is an effective approach to supply chain performance improvement.

1078

MATERNAL AND NEONATAL MORBIDITY IN SOUTHERN PROVINCE, ZAMBIA

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There is limited understanding of the prevalence of maternal and neonatal morbidity in Zambia. In the context of a large neonatal survival trial (ZamCAT), we investigated the prevalence of maternal and neonatal health danger signs and associated socio-demographic characteristics. Data on antenatal and postnatal maternal danger signs including fever, vaginal bleeding, convulsions, swelling, severe headache, postpartum hemorrhage and breast pain were collected prospectively during home visits. Reported neonatal danger signs in the first 28 days of life included fever, hypothermia, jaundice, convulsions, omphalitis, diarrhea and difficulty breathing. Data were collected from 27,293 pregnant women and their neonates. 82.4% of the women were married; 10.2% had no formal education and 39.6% had completed primary school. HIV prevalence was 8.9%. Mean maternal age (± SD) was 25.6 ± 6.9 y and women had on average 3.6 ± 2.3 pregnancies. 2.95% had at least one danger sign during the antenatal and postnatal periods; most occurred on day 1 postpartum. The most common maternal danger sign at day 1 was excessive vaginal bleeding (0.64%). Women with at least one danger sign had a higher mean number of previous pregnancies (3.8 ± 2.4 vs. 3.5 ± 2.3 pregnancies, ($p<0.01$) and were older (26.3 vs. 25.6 years, $p<0.01$) . HIV-infected women were 1.52 times more likely (95% CI: 1.24, 1.87) to have at least one danger sign. 2.79% of neonates had at least one danger sign with highest prevalence at day 1 (1.35%), most commonly fever (0.47%) and difficulty suckling (0.63%). In contrast to the maternal health correlates, mothers of children with at least one danger sign had a lower mean number of past pregnancies [3.3 ± 2.1 vs. 3.6 ± 2.4 pregnancies) ($p<0.01$)] and were younger [25.1 vs. 25.7 years, $p<0.05$] compared to mothers of healthy children. Maternal HIV infection had no impact on the prevalence of neonatal danger signs. Danger signs occurred infrequently in pregnant women and newborns. HIV infection increased the risk of maternal morbidity but did not influence neonatal complications.

1079

DEVELOPMENT, VALIDATION AND INTERNATIONAL FIELD PERFORMANCE OF MULTIPLEX REAL-TIME QPCR ASSAY PANELS FOR THE DETECTION OF DIARRHEAGENIC VIRUSES, BACTERIA AND PROTOZOA

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With their increased sensitivity and decreased time to diagnosis, PCR-based diagnostics represent a significant improvement over to conventional microscopy, culture-based and ELISA-based methods. In this work, we developed and validated five TaqMan-based real-time qPCR assay panels for the detection of sixteen diarrheagenic pathogens including viruses, *E. coli* and non-*E. coli* bacteria, and protozoa. The validation process for all panels revealed at least five logs of linear range for each gene target with low to moderate CV values in within-run ("repeatability") and between-run ("reproducibility") precision tests. In addition, the assays perform well with stool extracts having varying degrees of inhibition. The assay panels were also evaluated at five international field sites using one of three real-time PCR platforms including the Bio-Rad CFX96, the Corbett/Qiagen Rotorgene, and the Applied Biosystems ViiA 7. Field evaluations were based upon the same linearity, repeatability, and reproducibility measures used during the validation process. While there were site-to-site differences for certain targets, the assay panels performed well over the five field sites, and importantly, similarly between the three real-time PCR hardware platforms. ROC curve analysis comparing the assay panels to conventional methods revealed that the assay panels have good predictive value. Future work will entail defining the Ct values that are predictive of a pathogen being associated with diarrhea versus asymptomatic carriage.

1080

WHY THE PRIVATE SECTOR IS A VITAL PARTNER IN SCALING UP DIARRHEA TREATMENT: LESSONS LEARNED FROM NEPAL, PAKISTAN, BENIN, AND GHANA

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Diarrhea is a leading cause of child deaths worldwide. Secondary DHS analysis of childhood diarrhea practices from 46 countries indicate a significant portion of caregivers from all wealth quintiles seek care from the private sector_ranging from 79% in South Asia and 66% in South East Asia to 50% in Sub Saharan Africa. In Nepal, Pakistan, Benin, and Ghana, USAID's POUZN and SHOPS projects developed public private partnerships to reach private providers and leverage private sector expertise: local manufacturers of quality affordable zinc/ORS assured distribution and marketing to retail providers through existing commercial channels; providers were trained in management with ORS and zinc; commercial advertising firms created compelling demand creation messages; and public sector partners assured an enabling policy environment for private sector engagement. Population-based household surveys were conducted in Nepal (n=3550), Pakistan (n=1713), and Benin (n=392) with caregivers of children under five with diarrhea to explore knowledge and practices following program implementation. All three countries showed increases in zinc use. Affordability, availability of zinc

products and recall of zinc messaging were important drivers of use. In Pakistan, there was a statistically significant association (p<.001) between exposure to a zinc message, either from media or a doctor, and use of zinc. In Benin, logistic regression analysis showed that recalled exposure to zinc messages, speaking to health personnel, seeking treatment from a health provider, and caregiver perceptions of zinc being effective and readily available increased odds of using zinc. In Ghana, mystery client surveys and interviews with providers showed increased knowledge and practices around prescription of zinc and ORS. Private sector sales of zinc tablets rose ten-fold after training and 300% after a media campaign. Together, this data shows the vital importance of engaging and leveraging the private sector in the introduction and scale up of zinc for childhood diarrhea treatment globally.

1081

AN EVOLUTION IN GLOBAL HEALTH INVESTMENTS: MOVING TOWARD SUSTAINABILITY

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Building on incredible progress against HIV/AIDS, tuberculosis and malaria, there is an evolution taking place in the world of global health delivery and financing. Many countries at the center of these epidemics - with the initial emergencies coming under control - are now able to scale up and take on an increasing level of responsibility for the health of their own citizens. The Global Fund to Fight AIDS, Tuberculosis and Malaria was founded more than a decade ago as an emergency response mechanism to address the three deadly epidemics. With approximately $23 billion in funding approved for more than 150 countries, the organization and its partners have helped to save 100,000 lives every month. As a result of the progress made to date - and to ensure that the fight continues in the most effective way possible - the Global Fund is evolving to become a catalyst for a more sustainable response to the three diseases. Friends of the Global Fight Against AIDS, Tuberculosis and Malaria recently completed a review of growing country capacity, analyzing the various ways implementing countries are strengthening health systems, building political will, and increasing domestic health investment. Using secondary research as well as a series of interviews from countries in Latin America, Asia, and sub-Saharan Africa, the resulting report identifies key trends, best practices and progress on the ground. This session, led by Deborah Derrick, President of Friends of the Global Fight Against AIDS, Tuberculosis and Malaria, will: provide an overview of the progress made against the diseases; outline the trends and best practices identified through research; articulate different ways sustainability can be measured - including, but not limited to domestic co-investment; highlight key country examples; and describe the roles of the various funding mechanisms - from multilateral and bilateral institutions to domestic and private sector investment.

1082

CHARACTERIZING HUMAN INTERACTIONS WITH PERIDOMESTIC RODENTS IN THAILAND: A PARTICIPATORY RAPID APPRAISAL

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Rodents are carriers and reservoirs of numerous pathogens that pose significant risk to humans, including many emerging diseases. In both urban and rural areas, peridomestic rodents (Family Muridae), such as rats and mice, pose a public health threat because they regularly come into contact with humans and domestic animals, providing opportunities for disease transmission. To prevent the spread of disease from these rodents to humans, feasible and acceptable strategies must be developed to minimize human exposure to rodents and their excrement. However, mitigation strategies will only be successful if they are framed within the context of the perspective, knowledge, skills and resources of a given community. To inform the development of mitigation strategies, we use rapid appraisal and participatory research techniques to characterize peridomestic rodent exposure among men, women and children in four sites in Khon Kaen province, Thailand, where peridomestic rodents are viewed as both a destructive nuisance and a source of food and income. The study characterizes peridomestic rodent exposure in these communities and identifies barriers to and opportunities for mitigating the risk of exposure and captures the continuum of people's experiences with peridomestic rodents - from 'opportunity' to 'mild annoyance' to 'serious threat.' Main themes explored in analysis are the location of peridomestic rodents, signs of peridomestic rodents, types of people who come into contact with peridomestic rodents, risks associated with interactions with peridomestic rodents, seasonal variations in exposure, the effects of peridomestic rodents on communities, perceived health impacts of peridomestic rodents, and mitigation strategies currently practiced in the communities. Factors such as gender, location (urban vs. rural, flood zone vs. non-flood zone), age, and occupation are considered. Based on study findings, several potential mitigation strategies are proposed.

1083

WHO IS AT HIGH RISK OF EXPOSURE TO AN EMERGING ZOONOTIC DISEASE? AN INNOVATIVE APPROACH TO CHARACTERIZING HUMAN-ANIMAL EXPOSURE IN HMONG AND LAO ETHNIC GROUPS IN LAO PDR

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A recent increase in emerging infectious diseases (EIDs) -- most of which are zoonotic - has been attributed to an increase in contact between humans and their domestic animals and wild animals. To help inform the design and implementation of interventions to reduce the transmission of EIDs, our study aimed to provide estimated rates of human exposure to different animals, quantify the risk of emerging infectious diseases, and identify populations or subgroups with particularly high rates of exposure. The population-based household survey among Hmong and Lao adult men (n=292), women (n=292), boys (n=203), and girls (n=189) was conducted in Khamkeut district, Bolikhamxay province, Lao PDR in March/April 2013. Independent samples were obtained for men and women aged 18-50 in each ethnic group; up to one girl and one boy aged 10-14 years of age were selected from every adult household that had children who met the age criteria. Animals of interest were wild animals particularly likely to carry zoonotic viruses -- bats, rodents, and primates - although the survey also collected information on other types of wild animals to which people were exposed. Selected domestic animals (pigs and chickens) were used as controls. A human-animal-interface assessment framework was developed to describe the relationship between different transmission routes, types of animal and human activities, and social and environmental factors influencing the emergence of zoonotic infectious diseases. Key indicators of contact related to different transmission routes were weighted to create an overall index of exposure to a specific animal. Simple and multiple logistic regressions were used to identify factors associated with being at "higher risk" of exposure to an EID. The main types of animal humans have contact with, and seasonal variations in specific human activities that put humans in contact with animals were assessed, and the frequency of exposure to animals and intensity of exposure were measured for each subgroup. This study is among the first to examine and quantify comprehensive human exposure to animals. It will help us understand how exposure to animals is mediated by social and environmental factors and identify interventions that might reduce risk of emerging infectious diseases in Lao PDR.

1084

RECOGNIZING MATERNAL EMPOWERMENT FOR IMPROVING UNDER-FIVE CHILD DIARRHEA IN INDIA

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India accounts for one fifth of global burden of childhood rotavirus mortality. With mothers being the primary care givers; their characteristics significantly influence child health. However, India has a gender inequality index of 0.62 ranking 129th (UNDP,2011) indicating a gap in female household decision-making. This analysis aims to understand the association between maternal empowerment (ME) and (i) the likelihood for a child to get diarrhea (ii) predicted rotavirus vaccination (RV) coverage nationally and in 6 regions (north, central, east, west, south, northeast). Estimates are based on National Family Health Survey III (2006) representing 19,138 children. ME Index comprising 11 variables (household decision making roles in social, economic, health domains) is created using Principal Component Analysis and categorized using quartiles. Indian Government plans to introduce a 2 dose RV hence the 2 doses of Diphtheria, Pertussis, Tetanus (DPT) vaccination are used as proxy. Logistic regression is used to predict associations, while adjusting for maternal education, settings, maternal employment and household wealth relative to the lowest ME quartile. Significant association is found between the highest quartile of ME with decreasing odds of child diarrheal incidence, (OR: 0.79, 95% CI 0.66, 0.94) and increasing odds of DPT2 coverage (OR: 1.29, 95% CI: 1.12, 1.47), nationally. Regionally, the odds of RV coverage is found to increase significantly in north (2nd quartile - OR: 1.72, 95% CI: 1.24, 2.40; 4th quartile - OR: 1.82, 95% CI: 1.25, 2.64), west (4th quartile - OR: 1.75, 95% CI: 1.11, 2.78) and south (3rd quartile - OR: 2.20, 95% CI: 1.50, 3.23 and 4th quartile - OR: 4.04, 95% CI: 2.65, 6.16). The odds of diarrheal incidence is found to decrease significantly in northeast (3rd quartile - OR: 0.35, 95% CI: 0.17, 0.72 and 4th quartile - OR: 0.48, 95% CI: 0.26, 0.90) and in south (4th quartile - OR: 0.53, 95% CI: 0.33, 0.85). Thus, region specific ME interventions in India could be highly impactful to improve child health and thereby reduce diarrheal incidence and mortality.

1085

BURULI ULCER OUTREACH EDUCATION: AN EXEMPLAR FOR COMMUNITY BASED TROPICAL DISEASE INTERVENTIONS

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Buruli ulcer is a neglected disease caused by infection of subcutaneous tissue with *Mycobacterium ulcerans* that can be treated effectively by a regimen of antibiotics if identified early, but which requires surgery if identified in its latter stages. In this presentation, we describe an innovative approach to BU education outreach as a means of increasing community awareness about the disease toward the end of early identification, decreased treatment delay, enhanced treatment adherence, and decreased treatment drop-out. The shortcomings of top down education approaches have been well documented. Education is never introduced into vacuum and needs to address existing perceptions/misperceptions, practices, and ways of knowing in a culturally sensitive manner. We describe the development and piloting of a BU education program based on formative research carried out in three West African Countries. The program utilizes a question:answer format and addresses issues of concern to both health staff and local populations encompassing BU signs/symptoms and ways of distinguishing BU from other common diseases, BU progression, perceptions of possible causes of BU, and treatment. The program is driven by a power point presentation that may be easily updated when new questions arise, and translated into local languages at community events. It also incorporates images explaining all actions taken by clinic staff in identifying and managing BU, and time series images of stages of BU healing so the public knows what to expect from treatment. Testimonials of former patients attest to quality of care such that the education program is driven by hope instead of fear. We present the steps taken in developing and piloting the intervention, the results of a three country evaluation, and data on people attending screening camps following the education programs. The program is offered as an exemplar for other tropical disease education programs.

1086

DO PROVIDER PERCEPTIONS OF MALARIA CASE MANAGEMENT PRACTICES MATCH ACTUAL PATIENT BEHAVIOR AT PRIVATE SECTOR DRUG RETAIL SHOPS IN NIGERIA?

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In Southwest Nigeria, even though most patients seek care for malaria from private sector pharmacists and patent and proprietary medicine vendors (PPMVs), little is known about provider treatment and patient care-seeking behavior for malaria case management. In 2012, we conducted qualitative research with pharmacists and PPMVs to assess provider perceptions of malaria case management. We then conducted a quantitative study of patient treatment-seeking behavior among patients purchasing anti-malarials from these types of retailers in Oyo State. Using a mixed methods approach, this study assesses the extent to which there is congruence between provider perceptions of customer demand and malaria case management preferences, and actual patient behavior at private sector pharmacies and PPMVs. Transcripts from 20 provider interviews and focus groups discussions were coded to identify domains of provider perceptions of malaria case management. These domains are then compared to survey data eliciting actual patient care-seeking behavior for malaria. We find that provider perception of malaria prevalence is much higher than confirmed malaria prevalence among pharmacy and PPMV customers (confirmed malaria prevalence was less than 5%). Providers perceived themselves as the primary malaria diagnosticians for their customers, but patients overwhelmingly reported diagnosing themselves (over 90%). Providers also expressed a desire to use rapid diagnostic tests for malaria, but patients reported a lack of trust for pharmacists or PPMVs to administer them. Providers perceived trust to be an important factor for where patients choose to seek care, which accords with reported customer preferences. Both providers and patients incorrectly associate many symptoms with malaria. These findings reinforce the importance of private sector drug retailers' role in malaria case management, but indicate that targeted interventions, including those to patients, may be necessary to change diagnosis and treatment behavior.

1087

IMPACT OF DISPARITIES ON THE POTENTIAL HEALTH IMPACT OF ROTAVIRUS VACCINATION IN NIGERIA: RESULTS OF AN INDIVIDUAL-BASED RISK MODEL

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Rotavirus diarrhea is a leading cause of child mortality in low-income countries, which could be reduced by new vaccines. We developed a model of rotavirus vaccine impacts for Nigeria, where mortality from diarrhea is high, and there are large disparities in vaccine access. We examined disparities in risk and vaccine access; how they affect vaccine impact, and how reducing disparities could improve health gains. We used the 2008-9 Nigeria Demographic and Health Surveys to develop an

individual model of diarrheal mortality and rotavirus vaccination. Child-level effectiveness was estimated based on whether children received DPT vaccinations, vaccination timing, illness age, and vaccination efficacy. A susceptibility index was developed from estimates of nutritional vulnerability and likelihood of diarrheal treatment to assess individual mortality risk for each child. The 20% of children with highest risk had 56% of the overall burden. Children in the poorest 20% of households had an estimated 35% of overall burden but only 8% of total vaccination effectiveness and 14% of the vaccination benefit. The estimated health cost due to these disparities is 46 times higher for children in the poorest as compared to richest quintile. Geospatial analyses display concentrations of risk and low vaccine effectiveness hotspots, located especially in the northeast and northwest regions of Nigeria. Disparities in coverage and effectiveness reduce vaccine impact, especially for the most vulnerable children. Reducing disparities in coverage could increase impact two-fold. Improving our understanding of disparities mechanisms could inform programmatic and policy decisions, improving health gains of vaccine introduction.

1088

SOCIOECONOMIC AND ENVIRONMENTAL DETERMINANTS OF CHILD DIARRHEA IN THE BRAZILIAN AMAZON

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Diarrheas are an important aspect of child health, especially in developing countries. The Brazilian Amazon is still an underdeveloped region, although some financial and social investments have been made by the Brazilian government throughout the decades. We analyzed the prevalence of diarrhea in children under 5 years of age in 2003 (n= 200) and 2010 (n = 377) in the same municipality in the Brazilian Amazon. A census was performed with mother of children living in the urban area of Assis Brasil, in the State of Acre, in the Western Brazilian Amazon, in order to determine the prevalence of self-referred diarrhea in the previous 15 days. Differences in prevalence were tested with Chi-square test or Fisher test. Diarrhea prevalence in 2003 was 34.5% in this age strata, decreasing to 18.2% in 2010 ($p < 0.001$, Chi-square test). In 2010, prevalence of diarrhea was higher in children between 12 and 24 months of age ($p = 0.01$) and in those attending baby care ($p = 0.063$). Socioeconomic variables associated with infant diarrhea were belonging to a household receiving governmental financial help ($p = 0.006$), indigenous ethnicity ($p = 0.047$), having a mother with very low income ($p = 0.062$), low schooling ($p = 0.01$) or who did not have a job in the previous 30 days ($p = 0.05$). Environmental factors were also associated with diarrhea. Living in houses made of wood ($p=0.022$), that were frequently flooded ($p = 0.039$) or that had an open sewage system ($p=0.06$), using a latrine ($p = 0.01$), not having piped water in the household ($p = 0.001$), electric power ($p = 0.011$), cooking stove ($p = 0.003$) or refrigerator ($p = 0.023$). The source and quality of drinking water was also a very important factor associated with diarrhea. Diarrhea was more frequent in children that did not have access to treated drinking water ($p = 0.004$) or mineral water ($p = 0.011$). Interestingly, adding chlorine, filtering or boiling the drinking water did not decrease the prevalence of infant diarrhea. In 2003, no variables were associated with diarrhea, probably to insufficient sampling. In this part of the Brazilian Amazon, infant diarrhea prevalence is high and still associated with socioeconomic features, quality of drinking water and household environment, which suggests an infectious etiology for it. Health promotion and investments in education and basic sanitation are still needed.

1089

ANEMIA IN PREGNANCY, BIRTH OUTCOMES AND CHILD HEALTH IN KENYA

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Anemia is common during pregnancy, particularly in resource-limited settings such as Sub-Saharan Africa. This study was conducted to examine the prevalence of anemia in pregnant women, and the relationship between maternal anemia, adverse perinatal outcomes, and child health in Kenya. Participants were 191 pregnant women enrolled in a prospective cohort study of perinatal health in Kisumu, Kenya. Binomial regression was used to estimate risk ratios and 95% confidence intervals, and multiple linear regression models were used to examine associations between maternal hematological status (hemoglobin, anemia (Hb<11.0g/dL), severe anemia (Hb<8.5g/dL) and adverse pregnancy outcomes, including low birth weight (<2,500 grams), preterm birth (<37 weeks gestation), and neonatal anthropometry. The average gestational age at enrollment was 20.8 weeks and the mean body mass index was 22.4 kg/m2. 50.5% of women had anemia and 7.9% had severe anemia at baseline, with an average hemoglobin concentration of 10.9 (± 1.7) g/dL. The mean birth weight of the children born to the participating women was 3,198.2 (± 437.0) grams with an average gestational age at birth of 39.2 (± 4.3) weeks. The incidence of low birth weight was only 5%; however, 18% of all children and 25% of the male newborns were stunted at birth (WHO length-for-age Z-score<-2). Maternal anemia was not associated with increased risk of low birth weight or other pregnancy outcomes. In conclusion, a majority of the women in this study had anemia during pregnancy; however, it was not associated with increased risk of adverse pregnancy outcomes. The incidence of low birth weight was lower than the national average though one in five children were born stunted.

1090

MALARIA AND NEGLECTED TROPICAL DISEASES IN PREGNANCY: RESULTS FROM A PROSPECTIVE PERINATAL COHORT STUDY IN KENYA

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Malaria and neglected tropical diseases remain a major threat to maternal and child health, particularly in Sub-Saharan Africa. In this analyses, we examined the prevalence of malaria and neglected tropical diseases in pregnant women, and their association with adverse perinatal outcomes in Kenya. Participants were 191 pregnant women enrolled in a prospective observational cohort study of perinatal health in Kisumu, Kenya. Binomial regression models were used to estimate risk ratios and 95% confidence intervals and linear regression models were used to examine associations between maternal infections and adverse pregnancy and infant outcomes. Parasitic infections (33%), including malaria (21%), pathogenic protozoan (20%), and helminth (11%) infections were common in pregnant women at baseline. Maternal malaria infection was associated with a five-fold increased risk of low birth weight (<2,500 grams; p=0.02); similar significant associations were observed for the outcomes of underweight (WHO weight-for-age Z-score < -2), wasting (WHO weight-for-length Z-score < -2), and BMI-for-age z-scores. In a novel finding, maternal malaria predicted a 1.44 cm decrease in head circumference at birth, equivalent to a reduction in head circumference-for-age by 1.14 (p<0.01). Pathogenic protozoan infections including *Entamoeba histolytica* and

Giardia lamblia were associated with a two-fold increase in the risk of stunting (p<0.01). Multiple intestinal parasitic infections were associated with a four times greater risk of stunting in male infants, compared to infants born to women with monoinfection or no infection (p<0.01). In summary, malaria and neglected tropical diseases are common in pregnancy in this population in Kenya. These infections severely compromise infant growth *in utero* and are associated with lower achieved weight, length, and head circumference at birth. A comprehensive approach to target infectious diseases in pregnancy is needed to reduce the risk of adverse perinatal outcomes in resource-limited settings.

1091

EPIZOOTIC MONITORING OF CLASSICAL SWINE FEVER IN POPULATION OF WILD BOARS IN THE TERRITORY OF UKRAINE

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We have conducted a retrospective epizootic, serologic, molecular and genetic monitoring of classical swine fever (CSF) in pigs in population of wild boars within Ukraine. When performing serologic monitoring, we used 3919 blood serum samples taken from boars shot off in hunting areas in 24 administrative oblasts of Ukraine during 2001-2009 hunting seasons. ELISA test-systems from IDEXX have been employed for the detection of antibodies to CSF virus in blood sera. 383 biological samples were studied to detect CSF virus RNA with Amplisens real-time PCR kits. The monitoring resulted in the revealing of 400 samples of positive blood sera. An average seroprevalence index was calculated at the level of 8.3 %. The seroprevalence indexes per hunting season were: 16.7 % in 2001, 36.4 % in 2002, 28.0 % in 2003, 7.9 % in 2004, 13.2 % in 2005, 8.8 % in 2006, 13.9 % in 2007, 6.0 % in 2008, 8.0 % in 2009, 6.4 % in 2010, 3.5 % in 2011, and 5.9 % in 2012. The serology study results indicated the presence and circulation of CSF agent in the wild boars population. It should be pointed out that the last CSF outbreak in wild boars was registered in Cherkassy oblast of Ukraine in 2002. RNA of CSF virus was detected by real-time PCR in one of 383 studied samples of biological materials. This sample was taken from a wild boar shot off in Kyiv oblast and analyzed in SRILDVSE. The obtained results of epizootic, serologic, and molecular-biological monitoring allow concluding the presence of CSF virus in the population of wild boars in the territory of Ukraine. This information allowed us to hypothesize that wild boar serve as a reservoir for CSF virus in Ukraine.

1092

FOLDSCOPE: ORIGAMI-BASED PRINT-AND-FOLD PAPER MICROSCOPE

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Here we describe Foldscope, a fully functional microscope printed and folded out of paper. Foldscope utilizes the principles of origami to implement bright field, multi-fluroscence, polarization, dark-field and projection microscopy. Foldscopes are manufactured in a roll-to-roll process where all the optical components including lenses, apertures and illumination electronics are directly printed on paper. Novel optical components including spherical GRIN lenses and integrated micro-apertures are also introduced. Panning and focusing are implemented in a single paper stage using a flexure mechanism. The entire microscope can be assembled in minutes, is extremely light (paper), packs in a completely flat configuration in a very small volume, operates with no external power, can be dropped from a 5-story building or stomped upon by a person, and can be incinerated if required. We hae established several potential applications of foldscope in ultra low cost "use and throw" microscopy for field diagnostics of diseases including malaria, chagas, giradiasis. Costing less than a dollar, Foldscopes can bring microscopy out of the lab and into the hands of citizen scientists enabling large-scale hands-on life science education opportunities.

1093

PREVALENCE OF CHRONIC KIDNEY DISEASE IN AFRICA: A SYSTEMATIC REVIEW AND META-ANALYSIS OF POPULATION BASED STUDIES

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Globally, Chronic Kidney Disease (CKD) has now been increasingly recognized as a major health concern. It is widely known as a major cardiovascular risk multiplier and can rapidly progress to end stage kidney disease requiring expensive treatments not limited to dialysis and kidney transplant. The burden of CKD in the developed world has been documented often in the medical literature; however, its prevalence in Africa remains unknown and unstudied. We performed a systematic review and meta-analysis of all the published evidence on the prevalence of CKD in population-based studies in Africa that use the standardized definition from the National Kidney Foundation Kidney Disease Improving Global Outcomes (KDIGO) practice guidelines. We conducted a comprehensive literature search of 11 electronic databases through April 2013 with no language restriction. We used the combination of various keywords relevant to CKD in our literature search and systematically reviewed published epidemiologic studies on the prevalence of CKD in Africa. We used a random effects model based on inverse variance method to estimate the pooled prevalence. We identified 644 potentially relevant citations and retrieved 64 full articles after title screening. Six studies published between 2008 and 2012 in five countries enrolling a total of 3936 participants whose mean age ranged from 28 to 54 years were included ultimately in the review. To estimate Glomerular Filtration Rate (eGFR) 2 studies used the Modification of Diet in Renal Disease (MDRD) equation. Three studies used both the MDRD and the Cockcroft-Gault (C-G) equation for eGFR while the remaining study assessed CKD using albumin creatinine ratio. The pooled prevalence of CKD by the MDRD vs. C-G estimating equations was 5% (95% CI: 3% to 8%) versus 16% (95% CI: 7% to 25%). Available evidence suggests that the MDRD equation tends to underestimate GFR in healthy individuals; in contrast, the C-G tends to overestimate. In conclusion, few population-based studies on the prevalence of CKD in Africa exist. Despite the difference in various estimating equations of GFR, the current body of evidence suggests significant prevalence of CKD in Africa, and further epidemiologic studies may be warranted for more accurate documentation.

1094

DENGUE IN INDIA: THE COST OF ILLNESS

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Between 2006 and 2012 India reported an annual average of 20,018 confirmed dengue cases. Although dengue has been a notifiable disease since 1996, regional comparisons suggest that reported cases does not reflect the full impact of the disease. A selective surveillance system, challenges in diagnosis, and paucity of economic studies all restrict policy makers' ability to understand the true burden of dengue and improve its control. We report findings from four-part national study to analyze the economic cost of dengue illness in India. Part one is estimating the cost of dengue hospitalizations to the Indian health system based on a retrospective study from 10 medical colleges across five regions of India. A randomly selected sample of 1448 medical records of hospitalized

dengue patients, between the years 2006 through 2011, were abstracted using a standardized questionnaire, obtaining length of hospital stay and other variables. Part two, a prospective study of 244 randomly selected ambulatory suspected dengue cases interviewed between October 2012 and March 2013, provides information on ambulatory services. Part three combines financial information, bed capacity, occupancy, and aggregate ambulatory visits in a macro costing approach to estimate the cost of a hospitalized bed day equivalent and an ambulatory visit. Part four, based on a case study in Madurai, a Delphi panel, and national surveillance data derives an adjustment factor for reported cases to project the true number of dengue cases nationally. Preliminary results suggest an average inpatient case cost $245.49 based on an average 7.09 day hospital stay and a cost per bed-day equivalent of $29.26 plus $38.04 for 4.4 ambulatory visits. An average ambulatory case cost $38.04 based on 1.6 visits to hospital outpatient department and 2.9 visits to other outpatient providers. Our expansion factor of 47.1 suggests that India experienced on average 943,000 confirmed dengue cases annually from 2006-12. Of these cases 282,900 (30%) were treated in the ambulatory setting with an average annual cost of $11 million, and 660,100 (70%) were hospitalized with an average annual cost of $162 million. The total cost for all confirmed dengue cases per year was $173 million. These preliminary data indicate that the economic burden of dengue in India is substantial and that control measures merit serious consideration.

1095

PATHWAY TO GROWTH FALTERING: COMPARISON OF GROWTH MODELING TECHNIQUES

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Growth is a common metric of the health status of a population, as it is relatively easy to measure and is associated with both short- and long-term health outcomes. Weight loss (or weight faltering) is known to be associated with acute insults (dietary insufficiency, infectious disease) whereas linear growth faltering is associated with cumulative, long-term burden of dietary insufficiency and infectious diseases. Wasting (weight-for-length Z-score <-2) and stunting (length-for-age Z-score <-2) have been associated with increased risk of mortality during childhood, and stunting has also been associated with lower educational attainment and economic productivity, as well as greater risk of chronic illness during adulthood. We will compare and contrast various methods to evaluate growth and growth faltering using data collected in eight countries using harmonized protocols as part of the MAL-ED cohort study. We will consider different approaches for looking at growth, including longitudinal models, splines, and continuous Z-scores, among others, and discuss the flexibility, interpretation, and merits of each approach for specific research questions.

1096

EFFECT OF ARTESUNATE TREATMENT ON *PLASMODIUM FALCIPARUM IN VIVO* GENOMIC EXPRESSION

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Artemisinin-based combination therapies are the main treatment for malaria in endemic countries. Both the mechanism of action and the mechanism of resistance to artemisinins are poorly understood. Transcriptomic studies can help in improving our understanding of these processes. During a prospective study of the efficacy of artesunate in monotherapy in children aged 1-10 years and presenting with uncomplicated *falciparum* malaria in Bougoula-Hameau, Mali, venous blood was collected before treatment (H0) and one (H1), two (H2) and three hours (H3) after treatment. RNA was extracted from these respective blood samples and used for microarray experiments with *Plasmodium/Anopheles* chips using the AffymetrixR platform. A total of 24 samples from 6 patients were included in the final analysis after quality control . For each patient differential gene expression was measured and expression levels at baseline (H0) was compared to levels at H1, H2 and H3 after treatment. Heat shock proteine 70, several ubiquitines and a number of AP2 domain-containing genes were significantly up-regulated in the immediate hours following artesunate treatment. We show that genes involved in cell cycle regulation, DNA damage repairs and response to heat shock were up-regulated in *P. falciparum* from patients treated with artesunate. Our data support a role for these genes in the *in vivo* response of *P. falciparum* to artesunate.

1097

STUDY OF VAR GENES EXPRESSION IN SEVERE MALARIA IN IMPORTED MALARIA CASES

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In the physiopathology of severe malaria (SM) caused by *Plasmodium falciparum*, the phenomenon of cytoadherence between parasitized red blood cells (PRBC) and cell receptors of the host plays a fundamental role. This interaction is mediated by PfEMP1, parasitic protein expressed on the surface of PRBC. Var genes encoding theses proteins are classified into several groups: A, B, C, B/A, B/C and var 1-4. Studies in the field demonstrated a relation between the var gene expression and the clinical presentation of the malaria case according to the age of the patients. The aim of this work was to study the expression of these var genes for the first time in imported malaria according to the ethnicity of patient (Caucasian, African immigrants and children of African immigrants born in France), the clinical presentation (SM vs. uncomplicated malaria (UM)) and the immune status of the host. A prospective study was done on imported malaria cases observed in metropolitan France and reported to the malaria national reference center in France. Epidemiological, clinical and biological data was collected for all included cases of SM and UM caused by *P. falciparum*. From each blood sample before treatment, DNA and RNA were extracted respectively for multiplication of infection (MOI) determination and for RT PCR to study var gene expression. Plasma was also collected for immunologic tests. Eighty malaria imported cases (31 SM and 49 UM) from 19 West African countries and with different ethnicities (14 Caucasian, 57 African immigrants and 9 children of African immigrants born in France) were included in the study. var gene expression studies by RT PCR revealed that var group A and B were more expressed in SM than in UM. This expression was also influenced by the ethnicity of the host: var group A was predominantly expressed in non immune patients (Caucasian and children of African immigrants born in France) during SM whereas var group B was predominant in African immigrants. These results were consistent with prior published data in studies of non immun children in endemic areas (Rottmann et al., Infect Immun, 2006). The immune status of the host seems to influence which var gene group is expressed and the occurrence of SM but this observation has to be further investigated.

1098

ELEVATED PHENYLALANINE LEVELS ARE ASSOCIATED WITH OXIDIZED BIOPTERIN IN TANZANIAN CHILDREN WITH MALARIA: A POSSIBLE BIOMARKER OF OXIDATIVE STRESS

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We previously reported hyperphenylalaninemia (HPA) in children with malaria. The mechanism underlying this metabolic anomaly is unknown. In healthy states, plasma phenylalanine (Phe) concentration is tightly regulated by substrate-level activation/inhibition of hepatocyte phenylalanine hydroxylase (PAH), the enzyme which mono-oxygenates Phe to tyrosine. A biopterin species, tetrahydrobiopterin (BH_4), is a required cofactor for PAH catalysis. PAH is activated as Phe levels rise and is inhibited by the product of BH_4 oxidation, dihyrobiopterin (BH_2). Under normal conditions, biopterin is maintained largely reduced as BH_4. We hypothesized that HPA in malaria reflects oxidative stress placed on the biopterin redox pathway and thus HPA would correlate with elevated BH_2 levels. We addressed this hypothesis by quantifying the oxidized (BH_2 and biopterin [B]) and reduced (BH_4) biopterin species in urine, the most accurate method available for measuring total body metabolism of these compounds. Children 6 months to 6 years of age presenting at two district hospitals in Dar es Salaam with cerebral malaria (CM, n=53) and uncomplicated malaria (UM, n=67) were prospectively enrolled. Similar aged, healthy children (HC, n=116) and children presenting with non-malarial CNS conditions (NMC, n=53) were prospectively enrolled as controls. Plasma Phe was measured by ion exchange chromatography. Biopterins were measured by HPLC with fluorescence and electrochemical detection on urine collected into redox stabilizer + chelator to prevent *ex vivo* oxidation. In HC 3/110 had Phe levels above the upper limit of the normal range (82uM), in contrast to 22/50 NMC, 46/63 UM and 38/50 CM cases. Phe levels varied significantly across the 4 groups (p=0.0001, Kruskal-Wallis). Of the 3 species of biopterins (B, BH_2, BH_4) and the reduced:oxidized ratio [BH_4:BH_2+B], plasma Phe correlated best with BH_2 concentration (r=0.53, p<0.0001, Spearman correlation). These data suggest that HPA in UM and CM may result from increased inhibition of PAH due to elevated BH_2 levels. Elevated BH_2 may arise when sufficient reducing power (NADH/NADPH) is unavailable in hepatocytes for recycling BH_2 to BH_4. An intracellular environment rich in oxidants might produce such conditions. Biopterin redox status may be uniquely sensitive to oxidation and thus HPA, while not specific to malaria, could serve as an easily measurable surrogate for oxidative stress.

1099

ERYTHROCYTIC CYCLE DURATION AND SYNCHRONICITY OF DIFFERENT *PLASMODIUM FALCIPARUM* CLONAL LINES

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Close correlation between the synchronous 48-hour cell cycle of *Plasmodium falciparum* and the human circadian rhythm suggests a link to virulence that depends on mechanisms of cycle duration and synchronicity of the asexual intraerythrocytic forms of the malaria parasite. The genetic determinants and molecular mechanisms regulating *P. falciparum* growth and development are not well characterized. Therefore, analysis of the relationship between cycle duration, synchronicity, and growth of the malaria parasites during the intraerythrocytic cycle will provide a deeper understanding of the underlying genetic mechanisms involved. We measured in triplicate the *in vitro* growth and synchronicity of three parasite strains every 8 hours over several cell cycles to build a precise compendium of these important phenotypes and their divergence across genotypes. Our data demonstrate that rate of synchronicity loss is a distinct feature of individual parasite clones: HB3 and 3D7 had similar cycle lengths and loss of synchronicity rates, while Dd2 had a significantly shorter cycle duration and lost synchrony more quickly. Our data suggest that the cultured parasite, without any cues from the host, has some control over its synchronous state. An Agent Based Model was generated utilizing these and previously published data to model intraerythrocytic parasite dynamics over time. These analyses suggest a relationship between cycle length and synchronicity that can be used to predict parasite growth, invasion efficiency and other phenotypes that influence virulence and interaction with the human host.

1100

FUNDAMENTAL INSIGHTS TOWARDS IN VITRO ANALYSES OF *PLASMODIUM*-HEPATOCYTE INTERACTIONS: PROTEOMIC COMPARISONS OF SUSCEPTIBLE AND REFRACTORY CELL LINES

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Our long-term aim is to reveal the full complement of proteins deployed by the exo-erythrocytic (EEF) liver-stages of human *Plasmodium* species and to understand how these parasites interact with host hepatocytes. We are especially interested in factors involved in cellular invasion, parasitophorous vacuole formation, and modulation of host cell death, as the ability to prevent cell death is likely a significant selective pressure on the parasite. Here, we address basic questions of cellular biology regarding a human cell line, HC-04, which is capable of supporting *P. falciparum* and *P. vivax* EEF development at low intensities. Using shotgun proteomics, and focused transcriptomics and immunohistochemistry, we have begun answering essential questions regarding (i) the optimization of *in vitro P. falciparum* invasion and development, (ii) HC-04 transcriptomic and proteomic profiles in the context of apoptosis vs. proliferation, and (**iii**) the glycoproteomic analysis of HC-04 relative to human hepatocyte lines that do not support the development of human malaria EEF stages. Comparative analyses or our results identify putative players in *Plasmodium*-hepatocyte interactions. This effort also lays a framework for more directed experiments, including ongoing analyses involving the effect of *Plasmodium* proteins ectopically expressed in human hepatocytes.

1101

MULTICLONAL ACTIVATION OF HYPNOZOITES IN RETURN TRAVELERS SUFFERING *PLASMODIUM VIVAX* RELAPSE

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In endemic areas, *Plasmodium vivax* relapses are difficult to distinguish from new infections. Genotyping of travelers who suffer relapse after returning to a malaria-free area can be used to shed light on the nature of hypnozoite activation and relapse. To examine multiplicity of infection (MOI) in relapsing *P .vivax* infections, we applied three different genotyping methods - microsatellites, heteroduplex tracking assay (HTA), and Ion Torrent amplicon deep sequencing - to recurrent *vivax parasitemia*s in two travelers who returned to Canada after acquiring

vivax infection overseas, one in Pakistan and one in Honduras. In both travelers, multiclonal relapsing parasite populations were found. While the HTA, which assessed MOI based on sequence variation at the merozoite surface protein 1 (*pvmsp1*) gene, revealed 2-3 parasite variants at each of the 5 relapse episodes, microsatellite analysis only revealed multiclonal infection in 3/5 relapse episodes as measured by the maximum number of alleles detected at 4 neutral microsatellites markers. In one traveler, the HTA revealed a unique hypnozoite variant at second relapse that was not detected at first relapse. Aside from this, variants at consecutive relapses were genetically identical. These results highlight the propensity for multiple hypnozoite clones to activate simultaneously to cause relapse. Results from Ion Torrent sequencing of *pvmsp1* will be used to delve deeper into the degree of polyclonality found at relapse. In particular, we are interested in whether parasite variants in the same person change in frequency with consecutive relapses, reflecting perhaps different propensities for hypnozoite dormancy, reactivation, or immune evasion.

1102

PHENOTYPIC CHARACTERIZATION OF PERUVIAN *PLASMODIUM FALCIPARUM* HRP2 NEGATIVE ISOLATES: A PRELIMINARY STUDY

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In 2010, *Plasmodium falciparum* wild isolates lacking HRP2 protein were found in Peru. Since then, several reports had published the distribution of these parasites in different countries and their implication in the performance of malaria RDTs. Although the function of HRP2 protein is unknown, this unique featured lead us to the study of the phenotype of these isolates in terms of parasite multiplication rates, invasion profile and characteristics associated with virulence. Two groups of isolates were evaluated and adapted to culture: Four HRP2 negative isolates and four HRP2 positive isolates. All isolates were evaluated for parasite multiplication rate (PMR), invasion profile, rosseting formation, adhesion to CSA protein and HUVEC cell line. *P. falciparum* strains, HB3, Dd2, 3D7, FCR3, ITG and CS2 were used as controls in the correspondent assays. PRM values were similar between HRP2 negative isolates (4.1± 2.5) and HRP2 positive isolates (5.0 ± 2.4). No differences were found for the invasion profile between both groups. None of these parasites were able to form rosettes. Only one HRP2 positive isolate was able to interact with CSA protein and only two HRP2 negative isolate were able to interact with HUVEC cell line. This is the first report that characterizes the phenotype *P. falciparum* HRP2 negative isolates and compares them with the phenotype of HRP2 positive isolates and laboratory strains. This preliminary study shows that the phenotype features of HRP2 negative isolates are very similar to the HRP2 positive isolates in Peru. Since HRP2 negative parasites are widely distributed, it is important to continue the study of the phenotypic characteristics of these parasites and whether or not their impact in the prevention, control or eradication measures against malaria that are now taking place around the world.

1103

NEAR-INFRARED FLUORESCENT IMAGING: A NOVEL TECHNIQUE TO ASSESS BRAIN PATHOLOGY AND SEVERITY IN EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria (CM) is a severe neurological complication of *Plasmodium falciparum* infection. It is characterized, in part, by severe cerebrovascular damage and inflammation, and deleterious effects on blood brain barrier (BBB) integrity. Matrix metalloproteinase (MMP) enzyme activity has been shown to strongly correlate with inflammation and has been studied in the context of several CNS diseases, including CM. We developed a novel imaging technique which allows *in vivo* investigations of brain pathology in our experimental model of CM (ECM). In the present study, we examined MMP activity and BBB structure and function during the course of ECM in mice infected with *Plasmodium berghei* ANKA (PbA), using near-infrared fluorescent (NIRF) imaging and compared our findings to mice with non-neurotropic severe malarial disease (*Plasmodium berghei* NK65) and uninfected mice. BBB disruption, was confirmed via quantification of Evans Blue extravasation, and MMP abundance and activity was verified by immunoblots and immunohistochemistry. NIRF imaging demonstrated significantly higher MMP activation in several brain regions in PbA mice, including in the brain stem, cerebellum, hypothalamus and cortex. This activation significantly progressed over time, correlating with increasing severity of disease, abnormalities in histological findings and the temporal onset of behavioral dysfunctions in ECM. In addition, *in vivo* imaging of the BBB demonstrated a progressive increase of BBB leakage in ECM mice, concomitant with the rise in MMP levels. Evans Blue quantification of *ex vivo* mouse brains corroborated our NIRF observations. Our data demonstrate that NIRF provides a sensitive and reliable approach to monitor the progression of ECM by allowing unprecedented direct visualization of neuroinflammation and BBB disruption, in a non-invasive manner, during the course of disease. This novel imaging technique may prove to be valuable in monitoring treatment of CM.

1104

HETEROLOGOUS *PLASMODIUM VIVAX* RECURRENCES DUE TO MINORITY VARIANT EXPANSION

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Malaria recurrence following *Plasmodium vivax* infection can be due to relapse, reinfection, or recrudescence. Molecular genotyping studies which have compared genotypes of initial and recurrent *vivax* infections often demonstrate heterologous recurrence (i.e. different genotypes than those seen initially), even in the setting of known relapse. We investigated whether heterologous relapse can be due to expansion of minority variants (variants present at less than 20% of the parasite population) originally present in the initial infection but undetected by standard genotyping methods. Using the Ion Torrent sequencing platform, we deep sequenced 133bp of the polymorphic *P. vivax* merozoite surface protein-1 gene in isolates collected from 69 Cambodians with *P. vivax*. In this cohort, 22 persons developed one or more recurrences (range: 1-3) a median of 68 days following treatment with dihydroartemisinin-piperaquine without primaquine. All samples were amplified and sequenced in duplicate, with mean 3,460x coverage. Sequences were defined as unique variants if they were present in both samples at ≥0.5% frequency and not determined to

be chimeras. We identified 50 unique *pvmsp1* haplotypes in the cohort. The mean multiplicity of infection was 3.7 (range 1-13 variants per person), with initial and recurrent episodes equally polyclonal. One-third of recurrences (9/29) had the same dominant or co-dominant haplotype/s at recurrence as seen in the preceding episode. Of the remaining 20 heterologous recurrences, nearly half (8/20) could be attributed to expansion of a minority variant seen in the initial infection. Reappearance, or outgrowth, of an originally minor population might occur randomly in a stochastic fashion, or it might occur if certain parasite variants are predisposed to hypnozoite formation and relapse. Variant "A", was responsible for half of the heterologous recurrences arising from minority variant expansion. However, it was also the most common variant overall, existing as the dominant variant in 9% of all initial infections and a minority variant 25% of the time.

1105

CLINICAL PROFILE OF MALARIA IN COLOMBIA AND THEIR ASSOCIATION WITH HOST IMMUNOLOGICAL STATUS

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Human infections by *Plasmodium* species have a broad clinical spectrum ranging from asymptomatic to severe malaria and complicated cases leading to death. The complex host-parasite interactions as well as geographic and social factors play a role in malaria pathogenesis. Although Colombia has one of the highest malaria incidences in the continent, few studies have documented the clinical profile and outcome of malaria infections in the country characterized by unstable transmission. One of the priorities of our NIH-ICEMR program is to generate a detail understanding of the clinical features of malaria infections in regions with different epidemiological conditions. We enrolled a total 1,600 individuals 1-80 y/age, attending outpatient clinics in Tumaco, Quibdó and Tierralta, three of the field sites of CLAIM, characterized by different epidemiological conditions, where both *P. falciparum* and *P.vivax* are prevalent with diverse transmission intensities. Participants provided a written informed consent/ascent and were subjected to clinical evaluation and laboratory tests (urine and blood) including malaria serology. Malaria cases were more frequent among young adults and housewives, students and fishermen. The majority of enrolled individuals (93%) presented with uncomplicated malaria. Few cases (n=3) of severe anemia (Hb <7g/dL) and severe thrombocytopenia (<20,000 ptt/µL) (n= 2) were observed, all caused by *P.vivax*. Mild anemia (Hb<11g/dL) was observed in 20% of cases and 48% displayed mild thrombocytopenia (<150,000/µL). Although, 11 individual presented high ALT levels (>120 U/L), no correlation with clinical manifestation was observed. High creatinine levels (>1.5 mg/dL) without any further renal manifestation were observed in 5 patients, three of them with *P.falciparum* malaria. Antibodies against *P. vivax* and *P. falciparum* blood stages were found in 60% and 27% of the studied population respectively, at low IFAT titers (≤1:80). The prevalence of IgG antibody reactivity to both PvCSP and PvMSP-1 was ~50%, most of them with titers ≤1:400. The high prevalence of uncomplicated malaria together with the low antibody titers appear to be associated with the low transmission intensity and may indirectly indicate an early diagnose and effective treatment and reflects the low mortality (~20 cases/2012) recorded in Colombia during the last few years.

1106

EVIDENCE FOR THE O-LINKED-β-*N*-ACETYLGLUCOSAMINE MODIFICATION IN *PLASMODIUM*

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The O-linked-β-*N*-acetylglucosamine (O-GlcNAc) is a dynamic post-translational modification, occurring in nuclear and cytosolic compartments, that has been shown to be an important mediator of stress response, intracellular signaling and cell survival among others. While O-GlcNAc has been well studied in Metazoans little is known about this modification and its role in the cellular biology of Protozoans, including apicomplexan parasites in the genus *Plasmodium*. The causative agents of malaria, *Plasmodium spp.*, undergo a very complex life cycle which includes development of the parasite within the host's red blood cell (RBC) and subsequent extracellular development of the parasite inside the mosquito vector. Both environments provide numerous stressors for parasite development, and to survive, it must rely on a very efficient defense response. If the O-GlcNAc modification is indeed present in *Plasmodium*, its role as a mediator of stress in other organisms suggests the potential for a similar role in the parasite. A close examination into the presence and possible roles of O-GlcNAc in malaria biology has not been adequately and properly performed. To date the enzymes responsible for the addition and removal of this modification remain unknown in *Plasmodium*, and previous studies in asexual stages have prevented the unequivocal demonstration of this modification due to the presence of contaminating host RBC O-GlcNAc modified proteins. We performed Western blot analysis using specific O-GlcNAc antibodies and proteins purified from ookinetes and sporozoites, both extracellular stages of the parasite, and confirm the presence of O-GlcNAc-modified proteins in *P. berghei* and *P. falciparum*. Addition of the modification onto parasite proteins was increased after drug treatment suggesting a role in stress response as hypothesized. Immunofluorescence assays of ookinete and sporozoite stages showed the presence of this modification in the cytosol of the parasite. Additionally, we have identified using mass spectrometry a subset of proteins with predicted O-GlcNAc modification sites. In an effort to identify the parasite OGT and OGA we used a bioinformatic approach to identify two candidate genes for OGT and one candidate gene for OGA. To test whether we identified putative homologs of OGT and OGA, we generated knockouts of the candidate *loci* in *P. berghei* and evaluated their role in O-GlcNAc modification.

1107

INVESTIGATING MECHANISMS OF PYRETHROID RESISTANCE IN FIELD POPULATIONS OF *ANOPHELES FUNESTUS* IN SOUTHERN AFRICA

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Mechanisms underlying insecticide resistance of *Anopheles funestus*, a major malaria vector, remain uncharacterized. In this study, WHO bioassays carried out on An.*funestus* from Mozambique, Malawi, and Zambia indicate that pyrethroid and carbamate resistance previously detected in southern Mozambique has spread northward while full susceptibility remains for organophosphates. Piperonyl butoxide (PBO) synergist assays indicated that a metabolic resistance mediated by cytochrome P450s is the likely cause of pyrethroid resistance. Microarray analysis identified three main P450 genes (CYP6P9a, CYP6P9b, and CYP6M7) which varied in expression levels between countries. Lower expression levels were observed for the duplicated CYP6P9a and CYP6P9b in central and northern Malawi and Zambia while CYP6M7 expression was higher in these locations. The up-regulation of these genes was validated by qRT-PCR. Using transgenic expression of these proteins we were able

to functionally metabolize both type 1 and type II pyrethroids but not carbamate. Other genes potentially involved in resistance are short chain dehydrogenases, ABC transporters (ABCB7), and other P450s such as CYP6AA4 and CYP9J11. To further investigate the genetic differences between these populations (N=10) we analyzed 48 field isolates from ten locations using 17 microsatellites spanning the genome. We observed a significant difference between populations at the geographical and temporal level (pairwise Fst score p≤0.05). These results parallel our microarray data and we are currently sequencing the rp1(6P9a/b) and rp2(6M7) Quantitative Trait *Loci* to pinpoint genetic differences the extent of selection in these important P450s and show how mechanisms of resistance vary between populations. Further ongoing analyses will help to establish a comprehensive picture of mechanisms controlling pyrethroid resistance in field populations of this important malaria vector.

1108

MITOCHONDRIAL DNA VARIATION IN *ANOPHELES PSEUDOPUNCTIPENNIS THEOBALD* (*DIPTERA: CULICIDAE*) FROM COLOMBIA, SOUTH AMERICA

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Anopheles pseudopunctipennis plays an important role as malaria vector in Central and South America. In Colombia, it is a suspected local vector and nothing is known about its population structure. This study used a 624 bp fragment of the mitochondrial Cytochrome Oxidase I gene to explore genetic variation in *An. pseudopunctipennis* natural populations collected in six localities of three Departments: Antioquia-A (*n*=21), Cordoba-C (*n*=23) and Valle del Cauca-V (*n*=33). Forty-four haplotypes and a high haplotype diversity for all populations (*Hd*=0.73810-0.97538) were detected. The nucleotide diversity was higher in C (*Pi*: 0.01638), followed by A (*Pi*: 0.00797) and V populations (*Pi*: 0.00607). Also, the highest average number of nucleotide differences (*K*= 10.20553) was detected for C. Significant negative values in the Tajima's D and Fu's *Fs* tests for the V population indicated possible population expansion or natural selection. While, C and A populations showed positive and no significant results. The *Fst* pairwise comparison between populations showed only significant differences ($p<0.05$) between Valle del Cauca and each of the others. The highest level of genetic differentiation (*Fst*=0.49941, N_M=0.50) was detected between the Cordoba and Valle del Cauca populations. These results indicate that the Cordoba and Antioquia *An. pseudopunctipennis* populations are genetically similar, while a different gene pool is present in Valle del Cauca with little or no current gene flow. This study shows genetic structure of *An. pseudopunctipennis* along its distribution range and constitute a first approach to the genetic study of this species in Colombia.

1109

HUMAN ENCROACHMENT ON FORESTS MAY INCREASE EXPOSURE TO NEW MOSQUITO SPECIES AND PATHOGENS EVIDENCE FROM ZIKA FOREST, UGANDA

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The steady increase in contact between humans and wildlife is brought about by human encroachment, destruction of natural forests and environmental changes. Mosquitoes get exposed to new hosts and pathogens; creating possibilities for new disease patterns. Therefore identification of blood meal sources is important to determine the interaction between hosts and vectors. In this work, engorged mosquitoes were collected in Zika forest (Uganda) for a period of 12 months, and abdominal contents sequenced for c*ytochrome oxidase subunit I* and *cytochrome b*. The sequences were subsequently blast searched in Genbank and the analyses revealed the presence of mammalian (86%), avian (13%) and amphibian-derived (1%) hosts. The first record of human origin blood-meals from *Uranotaenia* species in Zika Forest was shown. Earlier studies showed these species to feed exclusively on reptiles, amphibians, birds or domestic mammals. Taking of mammalian origin blood-meals puts the human and entire animal community at risk because of the possibility of exposure to new pathogens. Significant differences between host species were observed (Kruskal Wallis test, χ^2 = 19.118, df = 5, p = 0.018) suggesting a wide range of host exposure. This could possibly create new disease patterns. Several mosquitoes may be considered potential bridge vectors for a number of arboviruses from the composition of their blood-meals. These results highlight the public health significance of taking measures to avoid encroachment of forests and reserves for diseases prevention and control.

1110

REVERSE-GENETIC AND PHARMACOLOGIC STRATEGIES FOR PROBING MOSQUITO INWARD RECTIFIER POTASSIUM CHANNELS AS INSECTICIDE TARGETS

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Diseases such as malaria and dengue fever are transmitted through the bite of infected mosquitoes during blood feeding. Insecticides remain the most effective means to kill mosquitoes and prevent the transmission of these devastating diseases; however, the continued emergence of insecticide resistance in mosquito populations has reduced their efficacy. Thus, the development of new mosquitocides is critically needed to slow the spread of mosquito-borne diseases. Inward rectifier potassium (Kir) channels play key physiological roles in epithelial, nerve, and muscle cells in mammals. However, their functions and potential as insecticide targets in mosquitoes have remained largely unexplored. We recently showed that a small-molecule inhibitor of *Aedes aegypti* Kir1 elicits 'renal failure' in mosquitoes and leads to death. Since then, a high-throughput screen of more than 40,000 compounds led to the discovery of several additional small-molecule inhibitors of *Aedes* Kir1, and they are now being evaluated as potential insecticides. Furthermore, to begin elucidating the functions of Kir1 in *Anopheles gambiae*, we used RT-PCR analysis to reveal that Kir1 is expressed in several tissues, including the ovaries, suggesting that Kir1 may contribute to mosquito reproduction. To test this idea, RNA interference was employed to knock down the expression of Kir1. Pilot studies suggest that knock-down of Kir1 decreases mosquito fecundity. The use of these novel molecular and pharmacological tools will be useful in further evaluating the potential of Kir1 as an insecticide target in disease-carrying mosquitoes.

1111

CYTOGENETIC ANALYSIS REVEALED INVERSION POLYMORPHISM IN A MAJOR MALARIA VECTOR IN CENTRAL AFRICA, *ANOPHELES MOUCHETI*

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Anopheles moucheti is a primary vector of malaria in forested areas of Central Africa. A recent study conducted in Gabon suggests that

this species is a major candidate vector for the possible transfer of *Plasmodium* parasite from apes to human. Epidemiologically important adaptations and behaviors of major malaria vector in Africa, *An. gambiae* are associated with the polymorphism of paracentric chromosomal inversions. However, population genetic and cytogenetic studies on *An. moucheti* have not been performed. The current study is the first attempt to characterize polytene chromosomes in *An. moucheti* females collected in three locations in Cameroon. We demonstrated that ovarian nurse cells contain readable polytene chromosomes, which are suitable for all standard cytogenetic applications. The homology between 2R chromosomal arms of *An. moucheti* and *An. gambiae* was established by fluorescent *in situ* hybridization of six *An. gambiae* genic sequences. Positions of the probes on chromosomes of *An. moucheti* detected substantial gene order reshuffling between the two species. A population analysis revealed the presence of three highly polymorphic chromosomal inversions in *An. moucheti*. Two of the inversions are located on the 2R arm and one inversion is found on chromosome 3. The frequency of the heterozygotes in the populations for one or more inversions was 50%. The high level of the inversion polymorphism in *An. moucheti* may suggest a complex population structure and/or pattern of ecological adaptations and behaviors in this mosquito. Interestingly, populations of other major vectors, *An. gambiae, An. arabiensis, An. funestus,* and *An. nili* have significantly reduced inversion polymorphism in Central Africa. Our study lays a foundation for a more detailed characterization of inversion polymorphism in *An. moucheti* and will stimulate population genetic, taxonomic, and genomic studies of this neglected malaria vector.

1112

ROLE OF MIRNAS IN 'FINE-TUNING' THE *ANOPHELES GAMBIAE* IMMUNE RESPONSE TO *PLASMODIUM FALCIPARUM*

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The transcriptional response of *Anopheles gambiae* to *Plasmodium* infection has been well documented, identifying a suite of immune genes involved in the host defense response. In recent years, post-transcriptional regulation of gene expression by microRNAs (miRNAs) has garnered increased attention. miRNAs are small non-coding RNAs responsible for post-transcriptional regulation in a sequence specific manner. miRNAs are transcribed by RNA Pol II to form pri-miRNAs, cleaved by Drosha to form pre-miRNAs and then by Dicer-1 into their mature form (21-25 nt). Argonaute-1 (Ago-1) then guides the mature miRNA to target mRNA 3' untranslated regions. We have demonstrated that RNAi targeting of *ago-1* and *dicer-1* cause a reduction in *P. falciparum* infection intensity, most notably when targeting *ago-1*. This indicates that preventing miRNAs from regulating target genes decreases survival of the parasite, suggesting a possible role for miRNAs in regulating the *Anopheles* anti-*Plasmodium* immune response and other mosquito factors that influence parasite infection To further investigate the role of miRNAs, we used a custom designed miRNA microarray profiling the differential abundance of 195 mosquito miRNAs between naïve and *P. falciparum* infected *A. gambiae* midguts when the parasite is invading this tissue. Currently there are 67 *A. gambiae* miRNAs annotated in mirBASE and here, we identified 32 midgut expressed miRNAs of which one was significantly upregulated by *P. falciparum* infection. Bioinformatics analysis suggests that this miRNA potentially post-transcriptionally regulates several innate immune genes, including the anti-*P. falciparum* IMD immune pathway factor *Imd* and effector *apl1B*. Therefore, antagomirs, chemically engineered oligonucleotides that silence endogenous miRNAs, are used *in vivo* to confirm the role of the miRNA in regulating the anti-parasitic immune response. Our data suggests that miRNAs are required to 'fine tune' the transcriptional response to *Plasmodium* invasion.

1113

POPULATION GENETIC STRUCTURE OF THE MALARIA VECTOR *ANOPHELES FUNESTUS* IN A RECENTLY RE-COLONIZED AREA OF SENEGAL RIVER BASIN AND HUMAN-INDUCED ENVIRONMENTAL CHANGES

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Anopheles funestus is one of the major malaria vectors in tropical Africa. Because of several cycles of droughts events that have occurred during the 1970s, this species had disappeared from many parts of sahelian Africa, including the Senegal River basin. However, this zone has been re-colonized during the last decade by *An. funestus*, following the implementation of two dams on the Senegal River. Previous studies in that area revealed heterogeneity at the biological and chromosomal level among these recent populations. Here, we studied the genetic structure of the newly established mosquito populations using eleven microsatellite markers in four villages of the Senegal River basin and compared to another *An. funestus* population located in the sudanian domain. Our results presume Hardy Weinberg equilibrium in each *An. funestus* population, suggesting a situation of panmixia. Moreover, no signal from bottleneck or population expansion was detected across populations. The tests of genetic differentiation between sites revealed a slight but significant division into three distinct genetic entities. Genetic distance between populations from Senegal River basin and sudanian domain was correlated to geographical distance. In contrast, sub-division into the Senegal River basin was not correlated to geographic distance, rather to local adaptation. The high genetic diversity among populations from Senegal River basin coupled with no evidence of bottleneck and with a gene flow with southern population suggests that the re-colonization was likely carried out by a massive and repeated stepping-stone dispersion starting from the neighboring areas where *An. funestus* endured.

1114

GENETIC STRUCTURE OF *ANOPHELES (NYSSORHYNCHUS) GOELDII* (*DIPTERA: CULICIDAE*) IN THE BRAZILIAN AMAZON

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Specimens of the presumptive malaria vector *Anopheles goeldii* in the Nuneztovari Complex were investigated to test the hypothesis of population structure within the Brazilian Amazon Basin. Species identification was confirmed by sequencing for the mtDNA Folmer region and Bayesian Inference analysis was conducted with known samples of *An. goeldii*. Twelve microsatellite *loci* of specimens from seven locations in Brazil: Boa Visa and Iracema in northern Roraima State; Santana in Amapá State, north of the Amazon River; and Altamira, Itaituba, Mojú and Santarém south of the Amazon River in Pará State, were analyzed. Genetic variability was very high (RS = 20; HE = 0.805). There was deviation from HWE for 31.3% of single-locus tests due to heterozygote deficits, and linkage disequilibrium was not significant. Among populations, FST estimates ranged from 0.008-0.078 and were significantly different for 16/21 pairwise comparisons. Gene flow (Nm)

was >1 for all comparisons. There was no significant correlation between linearized FST and geographic distance. Effective population size (Ne) was moderately large for all populations regardless of model (linkage disequilibrium or heterozygote excess). STRUCTURE analysis detected three clusters; one was the most eastern locality, Mojú; the second was the northern localities, Boa Vista and Iracema, and the third was Altamira and Santana (Pará and Amapá States, respectively). Itaituba and Santarém in western Pará state consisted of admixed individuals belonging to all three clusters. Interestingly, Itaituba was also the site of multiple white and COI genotypes in the malaria vector An. *darlingi* (JEC and MMP, unpub.) and might be an Amazonian diversity hotspot. The factorial correspondence analysis indicated significant differences between Mojú and all other populations. Mojú is of particular interest because *An. goeldii* from this locality has been implicated as a local malaria vector for both *Plasmodium falciparum* and *P. vivax*. In summary, high diversity and significant population structure may contribute to phenotypic differentiation of *An. goeldii*, especially in Mojú, eastern Pará state.

1115

MULTIGENE PHYLOGENY OF MAJOR AFRICAN MALARIA VECTORS PLACES *ANOPHELES NILI* IN THE BASAL CLADE

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Anopheles gambiae, An. arabiensis, *An. funestus*, and *An. nili,* are among the major vectors of malaria in sub-Saharan Africa. *An. gambiae, An. arabiensis*, and *An. funestus* breed in temporal or permanent freshwater pools. However, *An. nili* breeds in slow-moving streams and large lotic rivers exposed to light. Phylogenetic analysis of vectors could be useful for understanding of association between evolutionary genomic changes and selective pressures from a malaria parasite on the immune systems of mosquitoes. In this study, we reconstructed a molecular phylogeny of major African malaria vectors and several outgroup species using 49 genes. These genes were evenly distributed throughout five chromosomal arms of *An. gambiae*. We identified orthologous sequences in the genomes of *An. nili*, *An. stephensi, Culex quinquefasciatus*, *Aedes aegypti*, and in the transcriptome of *An. funestus*. Phylogenetic trees were generated using neighbor-joining method from all individual genes and concatenated trees were obtained according to chromosomal arms in MEGA 5.05 program. Most of the trees obtained from individual genes were consistent, placing *An. nili* as the basal clade among the studied malaria mosquito species. Results were supported by high bootstrap values in concatenated phylogenetic trees generated separately for each chromosomal arm as well as individual trees. We conclude that the African *An. funestus* and Asian *An. stephensi* are the most closely related and most recently diversified lineages, while *An. nili* lineage split early from the other African *Anopheles* species and belongs to the basal clade. The multigene genome-wide molecular phylogenic approach could be useful in understanding the evolutionary relationships among malaria vectors.

1116

AN ANALYSIS OF *ANOPHELES'* OPSIN GENES IN RESPECT TO DIURNAL BEHAVIORAL VARIATION

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Within the range of vector behaviors, we understand little about mosquito photopreference and the molecular mechanisms governing vision-dependent behavior in vector mosquitoes. Investigations of the influence of photopreference on mosquito behaviors such as endophagy/exophagy and endophily/exophily will enhance our ability to develop and deploy vector-targeted interventions and monitoring techniques. Previous laboratory-based analyses by our group have revealed that diurnal variation in photopreference and illumination intensity preference differ between *Anopheles gambiae* and *An. stephensi*. We are employing qRT-PCR to assess transcriptional expression patterns of long wavelength-, short wavelength-, and ultraviolet-sensing opsins (i.e., rhodopsin-class GPCRs) spanning two consecutive diurnal cycles, in *An. gambiae* and in *An. stephensi*. Using RNAseq data from *An. gambiae*, we are extending our analysis to the assessment of stage-specific transcriptional expression of genes implicated in phototransduction in first and third instar larvae, as well as in adult males and females. These analyses will provide insights into correlations between expression of phototransduction genes and diurnal photopreferences in *An. gambiae* and *An. stephensi*, and into the ensembles of phototransduction-associated genes expressed during larval and adult stages in *An. gambiae*. Our results will provide insights into molecular mechanisms involved in critical aspects of mosquito vision and the differential deployment of phototransduction machinery relevant to a number of vector behaviors.

1117

DYNAMICS OF KDR AND SPECIATION ISLAND INTROGRESSION BETWEEN *ANOPHELES GAMBIAE* MOLECULAR FORMS IN SOUTHERN MALI

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Anopheles gambiae is the most important vector of *Plasmodium falciparum* malaria. Due to control failures such as emerging insecticide and drug resistance, genetically modified malaria-resistant mosquitoes are seen as a potential new method for malaria control. Malaria-resistant *An. gambiae* have been engineered in lab colonies, but techniques still need to be developed to release GM mosquitoes and drive anti-*P. falciparum* effector genes into wild *Anopheles gambiae* populations. *An. gambiae* has significant population structure, and reproductive barriers may hinder the spread of introduced genes, or require development of multiple strains for release into different populations. Within *An. gambiae*, the M and S molecular forms appear to be differentiated by three divergent speciation islands on the X, 2L, and 3L chromosomes. Although not physically linked, the speciation islands are nearly always found together, indicating that hybrids are selected against. Recent data shows that ecological conditions may lead to breakdown of reproductive barriers between the forms, resulting in brief periods of hybridization and introgression. Using a panel of SNPs located in each speciation island and the *kdr* insecticide-resistance allele, we genotyped ~700 *An. gambiae* samples from southern Mali, over a period spanning a hybridization and subsequent introgression event. We were able to track introgression of the *kdr-w* resistance allele from the S form into the M form. The *kdr-w* SNP is physically linked to the 2L island, but selection for the *kdr-w* allele was strong enough to overcome selection against hybrids, causing the S-form 2L island to introgress into the M form. These data indicate that despite strong reproductive barriers, advantageous *loci* are capable of introgressing between population groups during brief bursts of hybridization. Besides describing how alleles such as insecticide resistance can move between populations, this natural example of gene drive may help to develop effective methods to introduce engineered anti-malaria genes into field populations.

1118

BEHAVIORAL SWITCHES AND GENE REGULATION IN THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

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The yellow fever mosquito *Aedes aegypti* displays considerable behavioral and physiological changes after acquiring a blood meal. Unfed females actively seek humans, but suppress this behavior after blood feeding, and switch to looking for oviposition sites within 48 hours. These changes are expected to be correlated with the expression of the olfaction genes that play a crucial role in these behaviors. Over two hundred olfaction related genes have been identified in *Ae. aegypti*. To examine which of these may play a role in host seeking, we peformed RNA-seq to quantify changes in the expression of olfaction gene mRNA in the main olfaction organ of *A. aegypti*; the antennae. Four-day-old females were blood fed and antennae were collected 0, 3, 24, 48 and 72h after feeding. Four functional groups of genes were analyzed: Olfaction Receptors (Ors), Ionotropic Receptors (Irs), Odorant Binding Proteins (Obps), and Gustatory Receptors (Grs). Most of the Obps are highly expressed in the antenna of females, whereas most Grs are absent. The Irs are generally expressed at low levels. In contrast, many ORs are expressed at intermediate levels. The expression profile of Obps and Irs does not change remarkably post bloodmeal, compared to the Ors. Or46 and Or99 are only expressed in unfed females whereas Or49 is only expressed after feeding. Totally 14 Irs, 6 Ors and 6 Obps are upregulated after feeding, while 8 Irs, 4 Ors and 11 Obps are downregulated post feeding. To examine if miRNAs may play a role in the regulation of olfaction gene translation, we also examined the expression of miRNAs in female antennae 24h after feeding, revealing the presence of over 30 new miRNAs. We are conducting a bioinformatics and *in vitro* assay to determine the targets of these antennal miRNA genes. The discovery of miRNAs controlling key genes required for mosquitoes to complete their life cycle will not only help us to better understand the vectors' biology, but can also help the development of novel vector control tools.

1119

MINING *ANOPHELES GAMBIAE* MOSQUITO GENOME FOR RESISTANCE GENES AGAINST MALARIA

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In order to identify genes related to *Plasmodium* parasite infection in *Anopheles gambiae* mosquitoes, we narrowed the *A. gambiae* parasite resistance island (PRI) to five small genomic blocks using co-expression patterns of genomic blocks, followed by direct association studies between non-synonymous single nucleotide polymorphisms (SNPs) and *Plasmodium falciparum* infection in wild *A. gambiae* populations from Kenya. The *A. gambiae* adenosine deaminase (AgADA), fibrinogen-related protein 30 (FBN30), and fibrinogen-related protein 1 (FREP1) genes were determined to be significantly associated with the *P. falciparum* parasite infection, and their functions in mosquitoes for the *Plasmodium* parasite infection were confirmed by RNA interference knockdown assays. When the FREP1 gene expression was knocked down, the *P. berghei* infection prevalence rate in *A. gambiae* mosquitoes were strikingly reduced from about 80% to 30%, indicating that the FREP1 gene is essential for *Plasmodium* invasion and may act as a receptor of *Plasmodium* parasites.

1120

SERUM MARKERS OF SEVERE CLINICAL COMPLICATIONS DURING *PLASMODIUM VIVAX* MALARIA MONOINFECTION IN THE PERUVIAN AMAZON BASIN

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Plasmodium vivax is the most widely distributed malaria species worldwide and responsible for ~75% of malaria cases in the Americas. There is growing evidence of severe complications caused by this malaria species. To identify biomarkers of severe malaria presentation caused by *P. vivax* mono-infection, we conducted a case-control study in two major reference hospitals in the city of Iquitos, Peru. Plasma samples were collected at time of malaria diagnosis from 46 subjects with *P. vivax* malaria mono-infection (severe malaria) who met at least one of a modified WHO criterion for severe malaria and 54 control subjects who presented with uncomplicated *vivax* malaria (non-severe malaria). Seventeen cytokines, chemokines and growth factors were assessed by Luminex xMAP technology and anti-$PvMSP1_{19}$-specific IgG antibodies were determined by ELISA. We found a significant elevation in the levels of interleukin (IL)-1β, IL-6, IL-10, IL-12, IL-13, IL-17, granulocyte macrophage colony-stimulating factor, gamma-interferon, monocyte chemotactic protein-1 and tumor necrosis factor alpha in the plasma of subjects with severe malaria ($p<0.05$). No significant difference was found in the levels of IL-2, IL-4, IL-5, IL-7, IL-8, granulocyte macrophage colony-stimulating factor and macrophage inflammatory protein 1 alpha. In addition, the titers of anti-$PvMSP1_{19}$-specific IgG antibodies were not significantly different between cases and controls, suggesting similar degree of prior malaria exposure in the two groups. Receiver operating characteristic (ROC) curve analysis revealed that IL-6 (AUC=0.76; 95%CI: 0.6-0.8) and IL-10 (AUC=0.73; 95%CI: 0.6-0.82) best discriminated severe from uncomplicated *vivax* malaria and, respectively) after adjusting for age, gender, *parasitemia* and levels of anti-PvMSP1 IgG antibodies. Further validation of these markers may provide accurate surrogate markers of severe complication during *vivax* malaria in endemic areas. We are currently investigating the predictive capacity of other inflammatory molecules including markers of endothelial cell activation and superoxide dismutase.

1121

DETAILED KINETICS OF B CELL SUBSETS FOLLOWING SYMPTOMATIC MALARIA IN CHILDREN IN TORORO, UGANDA

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Little is known about the kinetics of B cell subsets following naturally occurring human malaria infection. Recently, atypical memory B cells (MBC) have been hypothesized to have a role in protection. To evaluate these kinetics, we enrolled 40 children 4-5 years of age from an ongoing cohort study in Tororo, Uganda, where malaria transmission is intense (EIR >300). We collected blood via fingerprick at the time of symptomatic

malaria (D0), during which children were treated with an ACT, plus days 3, 7, 14, and 28 following treatment. Whole blood was stained, lysed, and frozen for subsequent analysis of B cell subsets via 10 color flow cytometry. Plasma cell frequencies were elevated on D0 (median=6.1% of B cells, IQR=3.5-8.8%), and decreased by D7 (median=2.5%, IQR=1.1-3.9%, p<0.005 vs. D0). Therefore, the day of presentation with symptomatic malaria provides a good time point to sample plasma cells in the periphery, many of which have likely differentiated due to the acute infection. Atypical MBC frequencies increased significantly from D0 (median=9.15%, IQR=5.8-12.5%) to D28 (median=15.8%, IQR=10.2-21.4%, p<0.001). Subjects with recently documented asymptomatic *parasitemia* had higher frequencies of atypical MBCs D0-D28 (p=0.049). Subjects with D0 parasite densities <25,000/ul also had higher frequencies of atypical MBCs D0-D28 (p=0.002). These data suggest a possible association between higher frequencies of atypical MBCs and that they may have a role in protection against malaria. Subjects with the highest incidence of malaria in the prior year (≥7 episodes/yr, n=20) exhibited a transient drop in frequencies of innate-like MBCs (CD27+IgD+IgM+) at the time of acute malaria not seen in subjects with a lower incidence (p<0.005 on D0 and D3, normalizing by D7). The reasons for this drop in innate-like MBCs, the primary contributor to serum IgM, in subjects with the highest incidence of malaria is unclear but warrants further investigation.

1122

A *FCGR2B* VARIANT THAT REDUCES HUMORAL IMMUNE SUPPRESSION IS ASSOCIATED WITH INCREASED SERUM MALARIA-SPECIFIC ANTIBODY LEVELS

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Engagement of the FcγRIIb receptor by antigen-containing immune complexes is a critical negative regulator of host immune responses on monocytes, macrophages and B cells. Recent observations show that homozygosity for a *FCGR2B* mutation that dampens inhibitory signaling (I→T at amino acid residue 232) is associated with increased risk for autoimmune diseases, but is also associated with protection against severe malaria in Kenyan children. Protection against severe malaria in 232T/T Kenyan children may result from a more robust humoral immune response against blood stage malaria and/or enhanced phagocytosis, which may select for this mutation. This hypothesis is supported by observations that the 232T/T variant is highest in malaria endemic areas (7-11%) and low elsewhere (~1-2%). We found 232T/T frequencies of 9 and 18% in Hardy-Weinberg equilibrium in two malaria endemic Papua New Guinea (PNG) populations whereas 232T/T was 6% in a PNG highland population not known to be associated with malaria endemicity. All Papuan populations have significantly higher 232T/T and 232I/T frequencies than Caucasians from North America and Northern Europe (p<0.0001 to 0.006) indicating a potential founder effect. However, the 232T/T and 232I/T genotypes are more frequently found in one malaria endemic PNG population than in the non-malaria endemic highlands population (p=0.027) suggesting a role for malaria in maintenance of the 232T allele in this malaria-endemic Papuan group. Surprisingly, 232T/T adults (>=18yo) had lower antibody (Ab) levels to a panel of most *Plasmodium vivax* (Pv) *P. falciparum* (Pf) Ags compared to individuals with 232I/I and 232I/T adjusted for age (p=0.0019 to 0.0488). Regression models of the age-related acquisition of anti-Pv and -Pf Ab responses indicate that 232T/T diverge from 232I/I and 232I/T between 4-10yo (Pv) or 6-20yo (Pf). Enhanced phagocytosis of opsonized malaria-infected erythrocytes in subjects with 232T/T may sustain this mutation in populations with heavy malaria and other infection burdens.

1123

IS SEROLOGICAL CROSS-REACTIVITY PREDICTED BY GENE ORTHOLOGY? A PROTEIN MICROARRAY STUDY OF SEROLOGICAL CROSS-REACTIVITY BETWEEN *APICOMPLEXA*N PARASITES *PLASMODIUM FALCIPARUM, P. VIVAX* AND *TOXOPLASMA GONDII*

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Antibodies are known to be exquisitely specific recognition molecules, and dysfunction of this property may have serious consequences for immune function and autoimmunity. Nevertheless, cross-reactivity of a given antibody with two or more related antigens does occur. The rules that define cross-reactivity are not well understood but are assumed to relate to amino-acid sequence homology between two or more antigens recognized by a given antibody. To examine cross-reactivity experimentally we constructed protein microarrays displaying proteins from three apicomplexan parasites: *Plasmodium falciparum*, *P. vivax*, and *Toxoplasma gondii.* Orthologous genes were identified using the *'Transform by Orthology'* tool implemented in EuPathDB (eupathdb.org). This uses the OrthoMCL database in which orthologs are defined by a combination of phylogeny-based, evolutionary distance-based and BLAST-based algorithms. Thus, *P. falciparum* and *P. vivax* share 5,259 orthologous genes, while *T. gondii* shares 3,114 and 2,980 orthologs with *P. falciparum* and *P. vivax*, respectively. Protein microarrays were probed with sera from human malaria cases from Mali (exclusively *P. falciparum*) and Peru (predominantly *P. vivax)* and from toxoplasmosis cases and controls from western Turkey (the only *P. vivax* cases in Turkey are in the east). In addition to detecting specific antibodies as expected, *P. falciparum* arrays detected antibodies in *P. vivax* infections from Peru, and *P. vivax* arrays detected antibodies in *P. falciparum* infections from Mali. These results show the arrays are able to detect cross-reactivity. Interestingly, antibody cross-reactivity between *P. falciparum* and *P. vivax* was observed for both orthologous and non-orthologous proteins. In contrast, toxoplasmosis sera showed only weak reactivity to *P. falciparum* or *P. vivax* antigens and were distributed equally among the cases and controls. These data show that orthology alone is not a good predictor of serological cross-reactivity.

1124

DETERMINATION OF THE IDEAL TIME TO MEASURE CYTOKINE EXPRESSION IN CULTURED PBMCS FROM *PLASMODIUM FALCIPARUM* INFECTED PATIENTS FROM IQUITOS, PERU

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Peripheral blood mononuclear cells (PBMCs) are used as an *in vitro* model for immunogenetic research with the stimulation of antigens. Different protocols use different incubation periods assuming that the dynamic in time of cytokines expression is similar. Theoretically, cytokine expression begins 6 hours after stimulation, but each cytokine expression vary. This study aimed to know the incubation period needed to obtain the highest cytokine expression in PBMCs non-stimulated and stimulated with MSP1 from malaria patients and endemic controls. Three cryopreserved PBMCs samples obtained by leukapheresis were used: one control (C), one asymptomatic (AS) and one symptomatic (S) patient. Each sample was

incubated at 37°C for 6, 10, 14, 18, 22 and 26 hours (h) after stimulation with MSP1 and non-stimulated. The cells then were collected for RNA extraction and cDNA synthesis. The mRNA from 4 cytokines was tested: TNF-α, IFN-γ, IL-10 and IL-12 by qPCR. The highest expression of cytokines was found between 6 and 14h in C and AS patients and between 14 and 26h in S patients. For Th1 cytokines (IFN-γ and TNF-α) the expression at 6 and 10h was similar between the S and the AS samples, being lower than the control and less expressed than the housekeeping gene. However, we found a high expression of IFN-γ and TNF-α at 14h in S sample with a relative expression of 10 and 4 folds higher than the control respectively. Compared to AS sample the relative expression of IFN-γ and TNF-α was 32 and 2.25 folds higher respectively. For IL-12, the relative expression at 6 to 18h is higher in the C than in patients, but at 26h the S is 2.25 folds higher than the others. For IL-10, the expression between 6 and 10h is higher in the C than in patients and between 14 and 26h S and AS expression is higher than the C, being more expressed at 18h in the AS. With these preliminary results we could infer that the ideal time to measure Th1 and Th2 cytokine expression in cultured PBMCs is within the first 14h or after 18h respectively.

1125

HOST AND PARASITE FACTORS UNDERLYING ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION IN MALIAN CHILDREN

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In Mali, *Plasmodium falciparum* transmission is seasonal during the 6-month rainy season, when almost all children below 8 years of age experience one or more febrile malaria episodes. On the other hand, during dry season virtually no children experience symptomatic malaria, even though ~50% carry blood-stage parasites. To determine the host and parasite factors that underlie asymptomatic infection, we performed a one-year longitudinal study of 580 children between 1 and 11 years of age. Subjects infected with *P. falciparum* by rapid diagnostic testing (RDT) at the end of the dry season were treated with standard anti-malarial drugs while RDT negative but PCR positive individuals for *P. falciparum* were left untreated; the third group included those who were uninfected at the end of the dry season. The risk of *P. falciparum* infection and clinical malaria was assessed prospectively during the subsequent malaria season and immune parameters were compared over time in the three groups. Individuals infected with *P. falciparum* at the end of the dry season who were left untreated showed a decreased risk of clinical malaria during the ensuing malaria season compared with uninfected individuals, and interestingly, treatment of asymptomatic infection at the end of the dry increased the subsequent risk of febrile malaria compared to the untreated group, indicating that chronic asymptomatic infection protects from febrile malaria. Analyzing polymorphic regions of *P. falciparum* genes we also compared the risk of super-infection in children whose asymptomatic infection at the end of the dry season was treated or not. In addition we are examining parasite factors that underlie chronic asymptomatic infection by comparing the metabolome and transcriptome of parasites collected from asymptomatic children *versus* parasites collected from children with acute febrile malaria. These data provide valuable insights into the host and parasite factors that underlie asymptomatic *P. falciparum* infection, as well as the risks associated with treating chronic asymptomatic *P. falciparum* infection.

1126

STUDIES OF *PLASMODIUM FALCIPARUM*-ASSOCIATED MODULATION OF THE HOST IMMUNE RESPONSE

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T helper cells play a critical role in orchestrating effective antibody responses, yet little is known about the CD4 T cell response to *Plasmodium falciparum* infection in humans. Recent data from animal models indicate that *Plasmodium* infection induces functional $CD4^+$ T cell exhaustion, and that reversal of $CD4^+$ T cell exhaustion leads to enhanced B cell and antibody responses and accelerates clearance of blood stage parasites, thus offering a potential explanation for the inefficient acquisition of protective antibodies in response to *P. falciparum* infection in endemic areas. In longitudinal studies in Mali in which PBMCs are collected before, during and after symptomatic and asymptomatic *P. falciparum* infections, we are testing hypotheses related to *P. falciparum*-associated modulation of the host immune response such as T cell exhaustion. In preliminary studies we observed that *P. falciparum* infection results in a higher frequency of CD4 T cells expressing programmed death-1 (PD-1), an inhibitory receptor associated with functional exhaustion of T cells. In ongoing work we are extending these analyses to understand the impact of acute and chronic *P. falciparum* infection on the phenotype and function of other components of the adaptive and innate immune system.

1127

MALARIA DURING PREGNANCY

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Malaria during pregnancy is a leading cause of maternal and infant morbidity, due to mother´s anemia, early birth and low birth weight. In Latin America, few studies address the subject of the immune response during gestational malaria, this is more notorious in *Plasmodium vivax* infection. The aim of the current study was to establish the effect of *P. vivax* infection in the balance of pro- versus anti- inflammatory cytokines and chemokines, and their relationship with some clinical and epidemiology outcomes. To this end, 43 pregnant women inhabitants in Uraba-South Cordoba. Out of these, 15 subjects were included at delivery (MGP+), 8 had history of gestational malaria (MGP-) and 20 had no exposition to infection throughout the pregnancy. Epidemiology and clinical data (age, gestational age, number of previous pregnancies, newborn´s weight, mother´s hemoglobin and parasitaemia) were recorded after reviewing the clinical records. At delivery, whole blood and plasma from the mother, placenta and cord, as well as placental tissue were collected. Diagnosis of infection was performed by thick smear and real time PCR. Pro-inflammatory ((TNFα, IFNγ, IL1β, IL17)/anti-inflammatory (IL4, IL6, IL10, TGF-β) cytokines and chemokines were measured by real time PCR and plasma level of them were assessed by multiplex ELISA. In order to characterize the changes on placental tissue (cell infiltrates, parasites, hemozoin, among other), histopathology analysis was performed and identification of the monocytes and NK cell infiltrates was carried out by immunohistochemistry. The clinical and epidemiology variables explored were similar in the three groups with exception of the gestational age. Placentas from the infected groups evidenced histopathology changes

including chronic villitis, fibrin deposition, intervillous inflammation, and hemozoin deposition. Monocyte and NK cells infiltrates were observed within the intervillous space and in villi itself. Cytokine expression showed a bias towards a pro-inflammatory status in both groups. Anti-inflammation cytokines remained unchanged. MCP1 was high in placentas of the MGP+ group and IL8 was high in the MGP- group. In conclusion, *P. vivax* induced modulation towards pro-inflammatory cytokines in placentas and produced histopathology changes that might affect the mother and fetus.

1128

ANTIBODY PROFILING IN INDIVIDUALS WITH *VIVAX* MALARIA LIVING IN TWO AREAS WITH DIFFERENT MALARIA ENDEMICITY AND DISEASE SEVERITY IN THE PERUVIAN AMAZON

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The development of naturally acquired immunity to *Plasmodium falciparum* malaria in endemic populations is believed to be affected by transmission intensity, age and parasite diversity based on studies conducted in high-transmission settings in Africa. However, very little is known regarding the acquisition of antibodies against *P. vivax*, particularly in areas of moderate to low malaria transmission. In addition, it is unclear whether individuals with different malaria clinical presentation differ in their anti-parasite antibody profile. To examine these issues, we measured the levels of IgG antibodies against ~500 *P. falciparum* and ~500 *P. vivax* seroreactive proteins (approximately ~10% of the malaria proteome) using protein microarray. We included individuals with PCR-confirmed *P. vivax* monoinfection who were enrolled in two areas with distinct malaria epidemiology and clinical presentation: Iquitos (IQT, n=20) and Madre de Dios (MDD, n=20), which are located in the Peruvian Amazon Basin. Among subjects from IQT, we included 9 cases who were hospitalized with *vivax* malaria and 11 non-hospitalized *vivax* malaria patients. Our preliminary results showed that the overall antibody reactivity to *P. vivax* proteins tended to be higher in individuals from IQT compared to those in MDD; about 3% of these differences reached statistical significance ($p<0.05$). Antibody responses against *P. falciparum* proteins were higher in Loreto where 10% the cases are due to *P. falciparum* malaria compared to Madre de Dios where no cases of *P. falciparum* have been reported in the last 10 years. Furthermore, uncomplicated malaria cases consistently presented higher levels of antibody against both *P. vivax* and *P. falciparum* compared to hospitalized malaria cases. Our results suggest that the degree of malaria endemicity, previous exposure to *P. falciparum* and potentially disease severity may influence the profile of anti-*P. vivax* antibodies generated during infection in low-to-moderate transmission settings.

1129

COMPARISON OF TRANSFORMATION, NORMALIZATION AND TESTING CHOICES IN A PROTEIN MICROARRAY ANALYSIS PIPELINE

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Protein microarrays have established an important role in the study of antibody responses to many tropical infectious diseases including brucellosis, malaria, melioidosis and salmonellosis. Papers have been published on appropriate methods for processing and analyzing protein microarray data, but few cover the entire procedure from start to finish or make fair comparisons among the various processing and analytical alternatives. We have categorized the processing of protein microarray data into three distinct steps: transformation, normalization and background subtraction. We also present several statistical testing options to compare the breadth and magnitude of antibody profiles. We then compare several transformation, normalization and statistical testing methods using previously published protein microarray data in which *Plasmodium falciparum*-specific antibody profiles were examined in a longitudinal cohort study in Kambila, Mali. We find robust linear model (RLM) normalization with generalized linear model tests of the antibody profile breadth to reveal the most powerful insights into the immune response against malaria. Going forward, establishing standard approaches to processing and analyzing protein microarray data will improve the rigor of antibody profiling studies and will facilitate cross-study comparisons.

1130

DIFFERENTIAL RESPONSE OF IFN-Γ USING DIFFERENT ANTIGENS IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF *PLASMODIUM FALCIPARUM* ACUTE MALARIA PATIENTS BY ELISPOT ASSAY: PRELIMINARY RESULTS

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This project has the aim to determine the production of IFN-γ by peripheral blood mononuclear cells (PBMCs) associated to clinical data in patients with *Plasmodium falciparum* acute malaria of different communities from Maynas, Peru. A total of 10 patients from rural (communities near to Nanay River) and peri-urban areas (Punchana and San Juan), and 3 endemic controls from an urban area (Iquitos) were tested in an IFN-γ ELISpot assay expressed by Spot-forming cells (SFC) per 2x105 cells. PBMCs were stimulated with 3 antigens: hemozoin (Hz), AMA-1 protein and merozoites (Mz). Hz and Mz were obtained from two different strains, ITG (referential strain) and F06 (wild isolate). We also used Hz DNase-treated as a control. We observed a difference between IFN-γ response to Hz ITG (23 SFC/2x105 PBMCs) and Hz F06 (26 SFC/2x105 PBMCs) versus Hz ITG DNase-treated (18 SFC/2x105 PBMCs) and Hz F06 DNase-treated (12 SFC/2x105 PBMCs) however there was no statistical difference. Mz ITG and Mz F06 were 68 SFC/2x105 PBMCs and 56 SFC/2x105 PBMCs, respectively. There was no statistical significance. The mean spots for AMA-1 protein was 3 SFC/2x105 PBMCs, very similar to the negative control. There was no significant difference between ITG and F06 strains in the IFN-γ response for Hz and Mz. We also reported a difference between genders in the IFN-γ production, men presented high levels of IFN-γ in Hz ITG, Hz F06, Mz F06 and AMA-1. Although Mz ITG presented a greater response for women, the difference is not significant comparing with men response. Furthermore, the *parasitemia*s (parasites/uL) were higher in men (12183p/uL) than women (3483p/uL). We only find a weak correlation ($R^2 = 0.4034$) between levels of IFN-γ and *parasitemia* in men, in which high concentrations of IFN-γ tend to have low levels of *parasitemia*. The 3 endemic controls did not present a significant IFN-γ response to the antigens. In conclusion, Mz presented the highest IFN-γ response in all studied patients followed by Hz. Unfortunately; AMA-1 recombinant protein presented the lower response of IFN-γ. Moreover, this study suggests that gender is a possible associated factor to the development of immune response and that IFN-γ production is related to *parasitemia* levels indicating that the immune system is acting against the parasite.

1131

PRELIMINARY EVALUATION OF CYTOKINES GENE EXPRESSION IN SYMPTOMATIC VERSUS ASYMPTOMATIC PATIENTS INFECTED WITH *PLASMODIUM FALCIPARUM* IN THE PERUVIAN AMAZON

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The balance between Th1 and Th2 cytokines seem to be crucial to survive an infection which requires the generation of a controlled immune response in the host, which recognizes and eliminates the invading pathogen limiting collateral damage to tissues, which may be an exacerbated immune response. The aim of this study was to evaluate the gene expression profile of pro-inflammatory Th1, regulatory cytokines and a chemokine in symptomatic versus asymptomatic *Plasmodium falciparum* infected patients from the Peruvian Amazon, in order to identify the cytokine pattern displayed by them during the acute disease. mRNA levels of Peripheral blood mononuclear cells (PBMCs) was analyzed in *P. falciparum* patients with different clinical outcome (Symptomatic, asymptomatic and control) which were cultured with different stimuli (Hemozoin (Hz), Hemozoin treated with DNase (HzTx), CpG and the MSP-1 recombinant protein). Within symptomatic individuals we have observed high levels of expression of TNF-α, IFN-γ and IL-12 (Th1 Cytokines) being predominant in PBMC's that were stimulated with Hz and moderate with HzTx. Likewise, TLR9 presented high levels of gene expression, the same pattern was observed when were stimulated with Hz and CpG. These levels of cytokine correlated with specific symptoms shown in each individual and *parasitemia* levels. These results were entirely different in the asymptomatic individual, finding very low levels of Th1 cytokines but presented moderate levels of RANTES gene expression when was stimulated with Hz but lower levels with HzTx. In this patient, we also observed high levels of IL-10 gene expression when was stimulated with MSP-1. In addition, other immunosuppressive activity related molecules were evaluated (CTLA4, FoxP3) and we found that the levels of expression of both molecules were lower in symptomatic compared to asymptomatic. In conclusion, we have observed that there is a characteristic cytokine expression profile that is involved in specific clinical status: Symptomatic samples presented high Th1 cytokines gene expression and asymptomatic presented moderate to high regulatory cytokines gene expression.

1132

OWNERSHIP AND USE OF INSECTICIDE-TREATED BEDNETS IN MACHINGA DISTRICT, MALAWI, SIX MONTHS AFTER A MASS-DISTRIBUTION CAMPAIGN

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Insecticide-treated bednet (ITN) mass distribution campaigns are being used to rapidly achieve ITN universal coverage and increase utilization..As part of the national mass distribution campaign in 2012, every household in six villages in Machinga district, Malawi, received one long-lasting polyester ITN for every two people in the household plus one extra for odd-numbered households. Existing bed nets were not taken into account in determining the number of ITNs to be given in the campaign. To evaluate the impact of the campaign on ITNownership and usage, two household censuses were conducted in February and November 2012, four months before and six months after the campaign. During each census, we collected data on bednet ownership and usage, sleeping spaces, demographics of household members and household characteristics. During the February and November census, 2,200and 2,657 households were censused and interviewed, respectively. Household ownership of at least one ITN increased from 70% (95% confidence interval [CI] = 68%-72%) before the campaign to 84% (95% CI = 83%-86%) afterwards. The averagenumber of ITNs per household increased from 1.1 to 1.8. Households with at least one ITN for every two residents increased from 23%(95% CI = 22%-25%) to 56% (95% CI = 54%-58%). ITN usage in all residents rose from 58% (95% CI = 57 - 59) to 70% (95% CI = 69 - 71). The percentage-point increase in ITN use was highest amongst school-aged children (between 5 and 15 years old),from 49% to 66%,whereas children aged <5 years increased from 71% to77% and adults >15 years increased from 59% to 70%. The mass campaign greatly improved ITN ownership and use although the goal of one ITN per two residents was not achieved. This could be because of the time lag between censuses and the campaign. Use of ITNs among school-age children continues to lag behind other age groups.

1133

TAKING LOCAL OWNERSHIP: GOVERNMENT AND HOUSEHOLD CONTRIBUTIONS TO INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL IN ZANZIBAR AND MAINLAND TANZANIA

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While foreign assistance has been instrumental in the initiation and implementation of large-scale malaria control efforts in Tanzania and other African countries, national government contributions are key to local ownership and sustainability. Indoor Residual Spraying (IRS) interventions were started in Zanzibar and Mainland Tanzania in 2006 and 2007, respectively, primarily through funding from the United States President's Malaria Initiative. This study aimed to explore in-kind contributions of the local government and households towards the total costs of IRS interventions. Data were collected through detailed interviews with local government officials involved in IRS and the technical team for the IRS project. Household contribution was estimated based on provision of water for IRS by households. Government contributions included government-provided warehouse and office space, fuel, vehicles and staff labor. In-kind cost contributions were analyzed and aggregated at the district, regional and national level. Calculations were based on proportion of total costs of IRS for the years 2008, 2009 and 2010. The cost per structure sprayed in Mainland Tanzania was $14.54 in 2009 and reduced to $14.23 in 2010. Zanzibar had a lower cost per structure sprayed; $9.78 and $6.92 in 2008 and 2010, respectively. On average, total in-kind contribution was 4.5% in Zanzibar and 3.0% in Mainland Tanzania. The proportion of government in-kind contribution was higher in Zanzibar versus the Mainland (3.4% vs. 1.3%) while household contribution was higher in Mainland Tanzania compared to Zanzibar (1.7% vs. 1.1%). As a proportion of total IRS expenditure, there was no increase in in-kind contribution in Mainland Tanzania or Zanzibar between 2008 and 2010. Government involvement, particularly through monetary transactions and increased in-kind contribution, needs to be encouraged for malaria control efforts to be locally owned, managed and sustained. To increase government and household contribution towards IRS, the government needs to become more centrally involved in project support, financing and capacity building.

1134

A COST-EFFECTIVE, PRACTICAL AND SCALABLE COMMUNITY-BASED MOSQUITO TRAPPING SCHEME THAT CAPTURES SPATIAL AND TEMPORAL HETEROGENEITIES OF MALARIA TRANSMISSION IN RURAL ZAMBIA

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Understanding factors contributing to spatial and temporal heterogeneity of malaria transmission at community level is important in determining control measures to apply. However, to promote sustainability and ownership of malaria control at community level, there is need for developing practically affordable sampling schemes. Community-based Centres for Disease Control and Prevention miniature light trap (CDC-LT) and the Ifakara Tent Trap model C (ITT-C) were compared with controlled quality assured (QA) human landing catch (HLC), CDC-LT and ITT in 14 clusters randomly selected for long lasting insecticidal nets alone or supplemented with organophosphate or pyrethroid -based indoor residual spraying (IRS). Cost and practical implication of the sampling methods were taken into account. Parasite and sporozoite rates were measured using trained local community health workers (CHW). There was no significant difference (p>0.05) in the mean catches of *Anopheles funestus* Giles by the community -based scheme (CBS) and the QA one. The crude relative sensitivity of ITT to the CDC light trap was 0.397. None of the methods captured more mosquitoes than the quality assured HLC indoor. Both HLC indoor and outdoor were equally efficient (Relative rate [95% Confidence Interval] = 1.104 [1.091, 1.210]).Parasite rates were variable over all the clusters but consistently high during the wet season with the organophosphate-based IRS clusters being lower than the rest. Similarly, *An.funestus* sporozoite infectivity rates followed a similar pattern. The CBS was relatively cheaper than the central level driven one. CBS systems could be affordable and reliable alternatives for surveillance of malaria in resource constrained malaria control programs. Even though HLC was more efficient than any other sampling tool, its ethical issues and laborious nature remain stumbling blocks for consideration at community. While both the parasite positivity rates and the sporozoite infectivity are consistently high in the wet season and in clusters which were not supplemented with organophosphate-based IRS, results in one cluster suggest this intervention requires periodic application to lower the saturated entomologic inoculation rate to levels were impact will be appreciated.

1135

ESTABLISHMENT OF AN *ANOPHELES DARLINGI* COLONY UNDER INSECTARY CONDITIONS THROUGH NATURAL COPULATION IN IQUITOS, PERU

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Anopheles darlingi is the main malaria vector in the Americas and among the most important in the world. Yet, very few studies describe its bionomics, parasite-vector relationships and vector competence mainly due to the lack of a laboratory colony. Important constraints limiting *An. darlingi* rearing have been the inability to stimulate copulation and oviposition in artificial environments, and the limited knowledge on optimal larvae rearing. We describe the establishment of an *An. darlingi* colony through manipulation of environmental conditions that promote natural copulation and the implementation of methods that effectively induce oviposition and reduce mortality. Adult females were collected in the village of Zungarococha, Department of Loreto, Peru, and taken to the NAMRU-6 Insectary in Iquitos where F0 offspring was reared. F0 adults (4,230) were placed in cages (60x60 cm) and stimulated to copulate naturally under a thermoperiod of 30°C during the day and 25°C at night and with a 30 min LED light stimulation period at dusk. Different oviposition containers were evaluated to optimize egg-laying. Larvae feeding regime and rearing conditions were standardized. We have used these conditions to successfully rear *An. darlingi* up to F3. An average of 36 sexual encounters/day/cage was observed in the first two generations ranging from 4-100 throughout a 5-day stimulation period. Spermatheca dissections (N=760 females) indicated an average insemination rate of 49% with the highest rate (95%) observed 8 days after stimulation. A total of 7,993 F1 eggs and 10,590 F2 eggs were obtained. F3 egg (11,000) and larvae (5,000) production is ongoing successfully. The average egg to adult mortality was 5% in the F1 (7,593 adults) and 63% in the F2 (3,873 adults). Pupae to adult mortality was low (0-4%) in both generations. Our novel, effective *An. darlingi* rearing methodology may allow critical studies of this important vector and could be a generalizable, cost-effective alternative to forced mating, a labor-intensive method generally used in anopheline laboratory rearing.

1136

HOUSEHOLD COSTS OF INPATIENT MANAGEMENT OF MALARIA IN MALAWI

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Little is known of the economic burden on households caused by costs of treatment for severe malaria. We present household costs resulting from inpatient treatment of severe malaria in Malawi, where 65% of the population live on less than $1 a day. We implemented a nationally representative cross-sectional survey of inpatient malaria management and costs. Thirty-six health facilities admitting malaria patients were randomly selected from a national list of hospitals stratified by managing authority and location. Researchers spent two days at each facility and interviewed all patients admitted for malaria within six hours prior to arrival. Patients being discharged on the day of interview were asked questions on hospital (consultation, laboratory tests, drugs and admission fees) and non-hospital costs (transport, accommodation and meals for patients and caregivers) incurred, before and during their admission. Costs incurred during previous health care visits for the same illness were included in the totals presented. A total of 82 patients were enrolled, of whom 46 (56%) were female and 42 (51%) were children under five years of age. The mean length of admission was 2.3 nights (range 0.5 to 12 nights). Nine (11%) patients reported seeking care at another facility prior to admission. Only 10 (12%) patients reported no expense during treatment of their illness. Of the remaining 72 patients, the overall mean expenditure was US$ 9.00 with a median of US$ 3.40 and a range of US$ 0.11 to US$ 144.81. Average hospital costs were higher (US$ 13.36) than non-hospital costs (US$4.57, p<0.011). On average, admissions at hospitals managed by the Christian Health Association of Malawi (CHAM) were more expensive (US$ 14.23) relative to those at hospitals managed by the government (US$5.23, p<0.010). Given the poverty level in Malawi, the inpatient treatment costs for severe malaria cases were high and potentially catastrophic to the majority of households. This study presents additional evidence and dimensions of the economic burden of malaria placed on poor households.

1137

FEASIBILITY, SAFETY AND EFFECTIVENESS OF COMBINING HOME BASED MALARIA MANAGEMENT (HMM) AND SEASONAL MALARIA CHEMOPREVENTION (SMC) IN CHILDREN LESS THAN TEN YEARS IN SENEGAL: A CLUSTER-RANDOMIZED TRIAL

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Current antimalarial strategies recommend (i) prompt access to diagnostic testing and treatment with effective antimalarial drugs such as Artemisinin Combination Therapy (ACT), (ii) intermittent preventive treatment for pregnant woman and infant (iii) Seasonal Malaria Chemoprophylaxis (SMC) and (iv)impregnated bed nets. Combination of antimalarial interventions can significantly reduce malaria burden. However, limited information is available on mechanism of combining antimalarial interventions. This operational research was conducted to assess the feasibility and effectiveness of introducing an integrated malaria control strategy, including HMM (using RDT, ACTs, Rectal artesunate) and SMC delivered by community health workers (CHWs). A cluster-randomised trial was carried out during 2 transmission seasons (2010-2011) in 8 villages located in the Southeastern part of Senegal. The intervention arm was represented by the combination of HMM+SMC while HMM represented the control arm. Children <10years of age were weekly followed by CHWs. At each visit, axillary temperature was measured and children with malaria suspected fever were RDT tested. Primary end point was the incidence of malaria attack over the follow up period. Secondary end points included: (i)malaria diagnostic accuracy (ii)access to ACT treatment (iii)SMC coverage (iv)safety and drug tolerability. The adjusted rate ratio comparing incidence of malaria attacks in intervention communities with control communities was 0.15, indicating a protective effect of HMM+SMC at 85%, (95%CI[21.2%-97.1%], p=0.005). RDT sensitivity and specificity respectively represented 89.2%(95%CI:70-100) and 86.2% (95%CI:70.2-100). A proportion of 96.4% of RDT confirmed malaria attack received AL. SMC coverage was evaluated at 97.3% (95%CI:91.3-100) in 2010, and 94.1% (95%CI [89.3-99.2] in 2011. No serious adverse event was noted. This study provided evidence that it is feasible to deliver SMC alongside an effective HMM intervention, while achieving high coverage and effectiveness of both SMC and HMM.

1138

MYANMAR ARTEMISININ RESISTANCE CONTAINMENT (MARC) SURVEY: MALARIA DIAGNOSIS AND TREATMENT

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The emergence and potential spread of artemisinin resistance in Cambodia, Thailand, Vietnam and Myanmar calls for immediate action from the global public health community to eliminate artemisinin resistant parasites from the region. One of the pillars of the Myanmar Artemisinin Resistance Containment (MARC) strategy is to ensure timely and effective case management of all malaria cases. However, limited epidemiological data available in Myanmar pose a challenge to monitor progress in stemming this global public health threat. In 2012, a malaria household survey was conducted in the areas of known and suspected artemisinin resistance (Tier 1 and Tier 2) to serve as a baseline for the MARC. The study domains included representative populations living in high to moderate malaria risk areas and utilized a multi-stage sampling approach stratified by Tier. In total, 1992 household respondents were interviewed using standardized and pre-tested questionnaires in line with similar malaria surveys previously conducted in Cambodia and Thailand. Overall, only 2.6% (95%CI 1.7 to 3.9) respondents would seek confirmation of malaria with microscopy or rapid test and 28.9% (95%CI 25.0 to 33.2) were able to name any specific antimalarial drug. According to the survey, the public sector was cited as the most popular source for test and treatment for malaria in both Tiers (66%); however, more in-depth analysis of this and a separate health facility survey are needed to better delineate public-private sources. Awareness of appropriate diagnostic services and treatment for malaria was insufficient in the MARC areas, and it is concerning that only a few respondents knew that some antimalarials were not recommended. Better targeted and innovative behavior change communications are needed to improve malaria knowledge and treatment-seeking behaviors amongst community members living in containment areas while also working with public and private (regulated and nregulated) providers to ensure provision of quality care for the diagnosis and treatment of malaria.

1139

A COMPARATIVE COST-EFFECTIVE ANALYSIS STUDY FOR ADOPTING UNIVERSAL MALARIA RAPID DIAGNOSTIC TESTS (MRDT) IN PEDIATRIC FEVERS ACROSS THREE SUB-SAHARAN AFRICA COUNTRIES

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In 2010, the World Health Organization issued a new guideline for malaria treatment. The guideline called for universal testing of malaria parasites prior to treatment in patients of all ages. Debates on whether malaria testing should be adopted for children under5 have divided malaria researchers. Economic evidence on the effectiveness of adopting such a strategy especially in settings with variable malaria transmission intensities remains sparse. This study explores whether adoption of Rapid Diagnostic Tests (mRDT) for malaria treatment in children under-five would be cost effective relative to presumptive treatment strategy in areas with variable malaria epidemiology across sub-Saharan Africa. We used Malaria indicator survey (MIS) data from three countries with different malaria transmissions to determine the effectiveness of the strategy. Additionally, country specific cost data on mRDT toolkits, drugs as well as health seeking behavior for children under5 and clinical practices were gathered from government agencies and also from published and unpublished studies. In cases where data were missing, experts' opinions were used. Data were uploaded in TreeAge decision tree software to determine the costs and cost-effectiveness of using mRDT relative to presumptive malaria treatments. Adoption of mRDT strategy for malaria treatment in children under5 was found to be cost effective across the three study countries relative to presumptive treatment strategy. However, the threshold for mRDT effectiveness was variable across the three countries. For instance, adoption of mRDT strategy in Angola was found to be highly cost effective compared to Uganda or Tanzania which had lowest prevalence cut-off points for the mRDT strategy being cost-effective. Local factors such as malaria prevalence, cost of testing supplies, the volumes of patients seeking care at primary health facilities and clinician's compliance were critical in determining the level of mRDT cost-effectiveness relative to presumptive treatment strategy. Universal formulation of malaria policies may not necessarily be cost effective. Policies should be tailored to reflect country specific malaria transmission status, clinical practices and local economic conditions.

1140

EXPLORING PATHWAYS THROUGH WHICH MATERNAL EDUCATION RELATES TO CHILDHOOD MALARIA INFECTIONS

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A number of studies have shown some strong relationships between maternal education child health and survivorship. There are very few studies that have systematically investigated the pathways and the interactions between maternal education and childhood malaria infections. We present evidence on the relationship between maternal education and childhood malaria infections based on cross-sectional malaria indicator survey data from three sub-Saharan Africa countries. We used pooled malaria indicator survey data from three sub-Saharan Africa countries and estimated multivariate logistic regression models to determine the relationship between maternal education and childhood malaria infections. We report the marginal effects for the probabilities of maternal education relating to childhood malaria infections. Additionally, we performed the Oaxaca decomposition analysis to quantify the contribution of maternal education on childhood malaria infections. The full adjusted model showed significant statistical association between maternal education and childhood malaria infection. Children under the age of five years within households where their mothers reported having some primary education level were 3.2 percentage points ($p<0.01$) less likely to have malaria parasites. Meanwhile, children of mothers with education level beyond primary school were 4.7 percentage points less likely to be malaria positive ($p<0.001$). The Oaxaca decomposition analysis of the full adjusted model exhibited a 7.8% gap in childhood malaria infection rates between educated and uneducated mothers. 60.8% of exhibited gap was largely explained by differences in household wealth, household place of domicile and differences in regional malaria transmission intensities. There was a significant statistical relationship between maternal education and childhood malaria infections across the three study countries. These findings provide additional evidence for support of malaria control policies focusing on integrated sector-wide approach for sustainable malaria control programs. Maternal education has huge potentials for sustainable long term reductions of childhood malaria infections reductions.

1141

TESTING MARKET-BASED AND REGULATORY STRATEGIES TO MINIMIZE OVERTREATMENT WITH ANTI-MALARIAL DRUGS IN ZANZIBAR

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Malaria burden in Zanzibar is at an all-time low, but the 2011 malaria indicator survey suggests only about one quarter of children receive blood tests for febrile illness, meaning that many who receive anti-malarials may not truly need them. Newly low-endemic regions like Zanzibar require strategies for ensuring that anti-malarial drugs go to those who truly need them. This study evaluated an intervention to improve rational use of anti-malarials in Zanzibar in which regulatory authorities implemented a policy banning sales of anti-malarials from over-the-counter (OTC) shops while subsidised malaria rapid diagnostic tests (RDTs) were introduced in private health facilities. Testing was already widely available in public health facilities. A controlled pre-post evaluation was used to assess whether the intervention changed the proportion of those with suspected malaria who sought care in OTC shops and of those that received parasitological confirmation. It also evaluated the impact of the regulations on the proportion of OTC shops selling anti-malarials. A midline evaluation was conducted six months after the baseline to determine the short-term effects of the intervention, and an endline evaluation was conducted a full year following implementation. Each evaluation consisted of 1,000 household surveys, 119 mystery client surveys, and 66 provider surveys. After six months, the intervention had a significant effect on the removal of antimalarials from OTC shops: the proportion of OTCs stocking antimalarials fell from 85% at baseline to 22% at midline (Chi2 Test $p<0.001$). However, the fraction of suspected malaria cases seeking treatment in OTC shops and receiving diagnosis remained unchanged at six months. The final evaluation conducted in April 2013 was analyzed to assess the impact of the intervention one year after regulations began, including qualitative investigation of patient perceptions of the new regulations. The results of this project suggest that government regulation of the private anti-malarial market is a viable path to encourage proper case management in an elimination setting.

1142

LOW-COST EDUCATIONAL TRAINING CAUSES SUSTAINED IMPROVEMENT IN PRESCRIBING AND STOCKING OF ARTEMISININ-BASED DRUGS IN MADAGASCAR

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Although artemisinin-based combination therapies (ACTs) are the recommended treatment for malaria in Madagascar, the 2011 malaria indicator survey indicated that fewer than 40% of anti-malarial drugs received by children under five for fever were ACTs. Approximately one third of suspected malaria is treated in the private sector where ACTs are often unaffordable or inaccessible. To encourage prescribing, stocking, and purchasing of ACTs in the private sector of Madagascar, educators were deployed to share scientifically accurate knowledge about ACT effectiveness with doctors and shopkeepers. Baseline cross-sectional surveys on factors related to prescription practices, anti-malarial stocking, and consumer drug preferences were conducted in 234 outlets and 163 health facilities in five regions of Madagascar in July 2011. Doctors and outlets in intervention regions were then visited by trained educators with ACT outreach materials between October 2011 and March 2012. Follow-up surveys in all regions were conducted in April 2012 immediately after the intervention concluded and again eight months later to evaluate longer-term effects. Midline results indicated that shops not stocking ACTs at baseline were 113% ($chi2=17.6$, $p<0.01$) more likely to do so if visited by educators, while doctors who had never prescribed them were 107% ($chi2=5.9$, $p<0.02$) more likely after receiving the educational visits. At endline, eight months after the visits ended, 93.8% of shops that began to stock ACTs and 92.3% of doctors who began prescribing them continued to do so. A 122% increase in anti-malarial market share of ACTs was observed in exit interviews with 406 customers at midline in both groups (27.7-60.4% intervention; 37-69.8% control), likely due to AMFm implementation in October 2010. Market share was similar at midline and endline (58.2% intervention; 69.7% control, n=391). These results suggest the potential for a short-term, low-cost intervention to cause sustained improvements in the availability of recommended drugs.

1143

DOCUMENTING MALARIA CASE MANAGEMENT COVERAGE IN ZAMBIA WITH A SYSTEMS EFFECTIVENESS APPROACH

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National malaria control programs and their partners must document progress associated with investments in malaria control. While this has been achieved through population-based surveys for most interventions, measuring changes in malaria case management practices has been challenging because the increasing use of diagnostic tests often reduce the denominator of febrile children who should be treated with an ACT - thus the indicator "proportion of fever cases in young children treated with the recommended antimalarial drug within 24 hours of onset" is less relevant. We examined an alternative sequence of indicators using a systems effectiveness approach and data from nationally representative surveys in Zambia: the 2012 population-based malaria indictor survey (MIS) and the 2011 health facility survey (HFS). The MIS measured treatment-seeking behavior among 972 children under five (CU5) and 1848 people age 5+ with recent fever. The HFS assessed case management of 435 CU5 and 429 people age 5+ with fever or history of fever seeking care at 149 health facilities. Consultation observation and exit interviews measured use of malaria diagnostic tests, ACT prescription, and patient comprehension of prescribed regimens. Systems effectiveness, using MIS and HFS data, was estimated as follows: 100% ACT efficacy x 47% fever treatment seeking from an appropriate provider x 68% blood testing x 88% ACT prescription for positive cases x 67% full patient comprehension of the drug regimen for patients prescribed ACT (proxy for adherence) = 19%. The largest gap in systems effectiveness in this context is low levels of treatment seeking behavior for fever. However, MIS results show that about half of febrile children who did not seek care from an appropriate provider had no evidence of infection (blood slide and RDT negative). Assembling MIS and HFS data to benchmark progress that can be attributed to investments in scale up of malaria diagnostics and treatment is feasible and can guide decision making to further improve malaria case management.

1144

MALARIA IN MPUMALANGA, SOUTH AFRICA: A MATHEMATICAL MODELING APPROACH TO UNDERSTANDING TRANSMISSION

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Malaria has been and remains a significant threat in Mpumalanga, a province on the Mozambican border of South Africa. As Mpumalanga is considered to be in the malaria elimination phase and the South African government begins to intensify efforts and commit scarce resources to decrease malaria incidence, there is a need to understand patterns in malaria transmission so that efforts may be targeted appropriately. Mathematical models have in the past provided a valuable framework for analyzing the dynamics of malaria transmission and are increasingly being used to test policy interventions so as to determine their impact on simulated transmission before implementing interventions in the field. Malaria transmission in Mpumalanga is seasonal from September to April, but transmission is still unstable and prone to sporadic outbreaks. The source distribution has changed in the last decade with 60% of reported cases being locally sourced in 2002, while at the end of 2012, 87% of reported cases had a foreign source; the vast majority originating from Mozambique. The season is characterized by three peaks where locally sourced infections and the northern most portion of the province contribute to the first peak (Spring) and foreign-sourced infections dominate the second two peaks (Christmas and Easter). While time series techniques showed rain lagged at 5 weeks to be significantly correlated with reported cases, multiple regression did not find rain as a significant covariate; a result that makes geographical sense given the trend of more foreign-sourced cases. An ordinary differential equation model of transmission is also used to validate these findings. Being able to understand the nature and source of malaria transmission will enable policy-makers to develop appropriate antimalarial strategies and this may lead to a better allocation of scarce resources and ultimately a greater impact on malaria.

1145

MODELING DEMAND FOR ARTEMISININ COMBINATION THERAPIES BASED ON EMPIRICAL RETAIL PRICES AND TREATMENT-SEEKING DATA FROM HOUSEHOLD AND OUTLET SURVEYS

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Global efforts to increase uptake of artemisinin combination therapies (ACTs) have focused on improving access and reducing the cost of ACTs across all distribution channels, most recently through the efforts of the Affordable Medicines Facility for malaria. However, reductions in the retail price of ACTs have had unclear effects on their uptake, and while broad distribution of ACTs has improved their accessibility, cheaper (though less effective) alternative antimalarials remain widely available. Since 2009, ACTwatch has conducted surveys at treatment access points in 8 sub-Saharan African countries: Benin, Democratic Republic of the Congo, Kenya, Madagascar, Nigeria, Uganda, Zambia, and Zanzibar. These biannual surveys collect volume and price data for all antimalarial medicines over the course of a week. For each treatment access point in the surveys, we calculated the fraction of antimalarial sales that were ACTs (mean <7% across all countries) and modeled this outcome as a function of mean ACT sales prices relative to mean sales prices of other available antimalarial drugs. By controlling for potentially confounding covariates (e.g., outlet type, access point accessibility, population density, and malaria prevalence), we modeled an empirical relationship describing how ACT market share changes with retail price. Results confirm that lower prices were associated with greater uptake. For example, ACT prices were 5.5x more expensive than non-ACTs in Nigerian shops that did not sell any ACTs during the 2011 survey period, but only 3.5x more expensive in shops that did sell ACTs. Applying the derived relationship to survey-derived estimates of demand for antimalarial drugs across sub-Saharan Africa yields estimates of ACT demand at different price points, providing information that drug manufacturers can use for production planning and giving policy makers a critical tool to shape the market dynamics of current and future malaria treatment interventions.

1146

INCREMENTAL COST-EFFECTIVENESS ANALYSIS OF INTRODUCING RAPID DIAGNOSTIC TESTING FOR MALARIA INTO REGISTERED DRUG SHOPS IN UGANDA

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Universal access to diagnostic testing for malaria followed by artemisinin-based combination therapy (ACT) for positive cases is now recommended by WHO. It is common in Uganda for people to seek treatment for malaria outside the formal health care sector often with drug shops as their first choice. Parasitological diagnosis to guide malaria treatment is not usually offered in drug shops. A recently finalised cluster-randomised intervention trial in Mukono District, Uganda, demonstrated that testing with malaria rapid diagnosis tests (mRDTs) in drug shops is feasible, can be associated with high provider compliance and resulted in significant increase in appropriate treatment compared to presumptive treatment. The incremental cost-effectiveness analysis of this intervention was evaluated using a decision analytical approach. Societal costs were collected for the two study arms. Provider costs incorporated the cost of community sensitisation, training of drug shop vendors, development of training material and the commodity costs such as the mRDTs used and the ACTs dispensed. Household costs of health care seeking were captured in a sample of drug shop customers who were interviewed in their homes after their initial visit to a drug shop. The purpose of these home interviews was to obtain all household cost incurred during a two-week period after the drug shop visit. These costs included out-of-pocket expenditure for travelling, fees, diagnosis and drugs for the first and any subsequent treatment visits as well as the opportunity cost of lost time. The effectiveness measure was 'correctly treated patient' defined as a research blood slide positive patient receiving an ACT or a blood slide negative patient not receiving an ACT. Effects were also translated into Disability-Adjusted Life Years lost. The incremental cost and effects of introducing mRDTs to increase appropriate ACT treatment in private, registered drug shops will be presented followed by sensitivity analyses incorporating factors like adherence, provider compliance and accuracy of the test.

1147

NOVEL LIVER STAGE ANTIGENS ASSESSED AS POTENTIAL VACCINE CANDIDATES AGAINST MALARIA

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The most attractive target for vaccine development against malaria is pre-erythrocytic liver stage (LS) of infection. The major barrier in developing vaccines is identification of antigens that will stimulate the most effective immune responses - a key for long-lasting protection. In this study we have screened and selected novel effective pre-erythrocytic vaccine candidates. On the basis of previously identified by microarray and RNA sequencing analyses *P. falciparum* (Pf) genes expressed exclusively during LS of infection, we selected *P. berghei* (Pb) orthologues of Pf genes and validated their expression by qRT-PCR in Pb infected C57Bl/6 (B6) mice. DNA constructs for 23 selected genes were cloned and tested for protective efficacy in B6 mice by Gene Gun, Electroporation and IM delivery. The efficacy of each DNA vaccine candidate was tested in a challenge model with CSP-DNA as positive control. Protective efficacy was measured by the reduction of Pb18S rRNA at 40h after challenge. 11 Pb LS antigens, administered as DNA vaccines, significantly reduced LS parasite burden in mice infected with Pb sporozoites and the level of reduction varied from 30% to 80% relative to vaccination with an empty vector. Two antigens sustained protection during 6 months after the last immunization. Moreover, three novel Pb LS antigens improved protective effect of CSP-DNA in our experimental model. Although we demonstrated that antibody responses induced by novel liver stage DNA antigens well correlated with the reduction of liver-stage parasite burden, depletion of CD8+ T cells resulted in loss of protective effects of DNA vaccines. Hence, our preliminary data suggest that protection induced by our novel pre-erythrocytic antigens was mediated by CD8+ T cells. This strategy allowed us to compare and prioritize new antigens according to their ability to induce partial protective immunity against malaria in mice. The protective antigens are being delivered by more robust vector platforms to achieve sterile protection and the results from these studies will be discussed.

1148

IDENTIFICATION OF NEW AND HIGHLY PROTECTIVE PRE-ERYTHROCYTIC ANTIGENS FOR MALARIA VACCINE DEVELOPMENT

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Malaria is the most devastating parasitic disease affecting humans. There is a great need for an effective malaria vaccine that could provide robust protection and contribute to the control and eventually eradication of malaria. Pre-erythrocytic antigens are particularly promising targets for malaria vaccine development with great potential to prevent infection and transmission, however, very few of these antigens have previously been identified and these are not sufficient to confer high levels of protection. Our vaccine rationale is based on the fact that immunization with radiation-attenuated sporozoites (RAS) or live sporozoites with chloroquine prophylaxis (spz + CQ) provide high level (~100%) protection against sporozoite challenge in both mice and humans, and this protection is dependent on the induction of CD8+ T cells targeting multiple antigens expressed in the pre-erythrocytic stages of the parasite life cycle. Our goal was to identify these novel antigens. Using a high-throughput genomics screening approach, we have identified some of the antigens that are targets of protective T cell responses in mice immunized with RAS and spz + CQ. Several of these antigens induced high levels of protection in outbred mice challenged with *Plsmodium yoelii* sporozoites. Our antigen discovery system utilizes an array of adenovirus vectors expressing 300 highly expressed *P. yoelii* pre-erythrocytic genes with identifiable *P. falciparum* orthologues. In the antigen discovery screen, antigen presenting cells were infected with individual adenovectors from the array and then mixed with splenocytes from mice immunized with protective regimens of RAS. We prioritized antigens based on the frequency of CD8+ T cell recall responses to each of the 300 antigens. We selected 50 antigens that recalled the most robust T cell responses and tested their capacity to protect mice from a *P. yoelii* sporozoite challenge. Outbred CD1 mice were immunized with a DNA prime - Ad boost regimen and sterile protection was measured following sporozoite challenge. Several of the prioritized antigens induced high levels of sterile protection. The *P. falciparum* orthologues of these antigens are being considered for advancement to clinical development.

1149

VACCINATION WITH SCHIZONT EGRESS ANTIGEN-1 PROTECTS MICE FROM *PLASMODIUM BERGHEI* ANKA CHALLENGE

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We discovered PfSEA-1 using a differential screening approach contrasting plasma from children who were resistant or susceptible to *falciparum* malaria. Antibodies to the immunorelevant region of PfSEA-1 (rPfSEA-1A, aa 810-1083) predict resistance to severe disease in two yr old children and block schizont egress from iRBCs. To evaluate the protective efficacy of vaccination with SEA-1 *in vivo*, we selected the *Plasmodium berghei* ANKA model based on its aggressive parasite growth rate, extreme lethality and the failure of known vaccine candidates (i.e. AMA-1 and MSP-1) to afford protection. We expressed and purified the *P. berghei* ANKA strain ortholog of rPfSEA-1A in *E. coli*, and conducted three vaccine trials in mice. Following vaccination, mice generated robust anti-rPbSEA-1A IgG responses in each trial. In Trial 1, BALB/c mice were vaccinated IP with rPbSEA-1A in TiterMax and challenged IP with 106 *P. berghei* ANKA iRBC. In Trial 2, C57BL/6 mice were vaccinated SC with rPbSEA-1A in TiterMax and challenged with 200 *P. berghei* ANKA sporozoites IV and in Trial 3, BALB/c mice were vaccinated IP with rPbSEA-1A in TiterMax and challenged IP with 104 *P. berghei* ANKA iRBC. In all three experiments, rPbSEA-1A conferred marked protection against a typically lethal *P. berghei* ANKA challenge as evidenced by up to 3.05 fold reduction in *parasitemia* seven days post-challenge ($P < 0.001$). Control mice required euthanasia by day 7-8 post challenge due to high *parasitemia* with associated morbidity, while none of the vaccinated mice had high *parasitemia* or overt morbidity. In addition, vaccination with rPbSEA-1A resulted in self-cure in 1/11 vaccinated mice in the first trial. These data constitute the first report of protection in *P. berghei* ANKA by vaccination with blood stage antigens and support our ongoing efforts to evaluate PfSEA-1 in the Aotus model of *falciparum* infection using both active vaccination with rPfSEA-1 as well as passive transfer of anti-rPfSEA-1A monoclonal antibodies.

1150

ANTIBODIES TO *PLASMODIUM FALCIPARUM* GLUTAMIC ACID RICH PROTEIN (PFGARP) INHIBIT PARASITE GROWTH BY ARRESTING TROPHOZOITE DEVELOPMENT

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In previous vaccine discovery efforts, we developed a differential screening method using plasma from children who were resistant or susceptible to *falciparum* malaria. Using this approach, we discovered PfSEA-1. Antibodies to PfSEA-1 predict resistance to severe disease in two yr old children, block schizont egress from infected RBC *in vitro*, and vaccination with rPbSEA-1A protects mice from *Plasmodium berghei* ANKA challenge. We have now adapted our differential screening method to field parasite-derived phage display libraries. In differential bio-panning assays, PfGARP (aa 411-673) was recognized by plasma pooled from resistant (n=11) but not susceptible (n=14) children participating in our birth cohort in Muheza, Tanzania. To further characterize PfGARP, we generated mouse antibodies against this immuno-relevant, highly invariant region (PfGARP-A) and performed growth inhibition (GIA) and immunolocalization studies. For GIA, 3D7 parasites were synchronized to the ring stage and plated at 0.3-0.4% *parasitemia* in the presence of anti-PfGARP-A or pre-immune sera (1:10 dilution). Parasites were cultured for 48 hrs and ring and early trohozoite stage parasites were enumerated. Anti-PfGARP-A inhibited parasite growth by 99% compared to controls ($P < 0.001$). In confocal studies, PfGARP localized to the RBC membrane in trophozoite and early schizont infected RBCs, but not to other parasite stages or uninfected RBC. To determine the mechanism of growth inhibition we performed trophozoite arrest assays (TAA) using anti-PfGARP-A. For TAA, 3D7 parasites were synchronized to the ring stage and plated at 5% *parasitemia* in the presence of anti-PfGARP-A or pre-immune mouse sera (1:10 dilution). Parasites were cultured for 36 hrs and trophozoite stage parasites were enumerated. Anti-PfGARP-A arrested trophozoite progression by 99% compared to controls ($P < 0.001$). These data support Pf-GARP as a novel vaccine candidate for pediatric *falciparum* malaria. By blocking trophozoite development, PfGARP may synergize with vaccines targeting hepatocyte and red cell invasion and schizont egress.

1151

DISCOVERY OF CONSERVED *PLASMODIUM* ANTIGENS ON THE SURFACE OF MALARIA-INFECTED RED BLOOD CELLS USING DNA APTAMERS

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Malaria continues to present a major human and economic challenge causing over 1.2 million deaths in 2010 and billions in lost economic potential. A vaccine targeting the leading causative agents, *Plasmodium falciparum* and *P. vivax*, would reduce much of the poverty associated with malaria and greatly assist in eradication efforts. Yet despite decades of work, no licensed malaria vaccine exists and some of the few candidates that have been evaluated elicit strain specific responses due to extensive polymorphism. During its intraerythrocytic stages (IE), *P. falciparum* remodels the host red cell membrane with a complex and poorly defined assortment of parasite-encoded proteins that undergo antigenic variation. Despite the requirement for immunologic stealth, exported parasite proteins also mediate strain-independent functions such as endothelial sequestration that are critical for pathogenesis. Based on this observation, we hypothesized that *P. falciparum* displays novel structurally conserved proteins on the IE surface and these proteins may serve as antigens for a broadly effective anti-malarial vaccine. In order to test this hypothesis, we developed an *in vitro* evolution technique that sequentially incorporates unique *P. falciparum* isolates as the target for Systematic Evolution of Ligands by EXponential enrichment (Serial-SELEX) to generate nucleic acid molecular probes, aptamers, capable of recognizing conserved surface determinants. Of the 11 aptamers identified, 10 demonstrated parasite-specific binding with nanomolar dissociation constants. Examination of the binding specificity of radiolabeled aptamers revealed a subset of aptamers that recognized all laboratory-adapted clones and clinical isolates of *P. falciparum* tested. Remarkably, these aptamers also recognized all tested laboratory and clinical isolates of *P. vivax* and *P. knowlesi* but not the murine malaria parasites, *P. chabaudi* and *P. berghei*. Competition studies showed that the aptamers bound a single target which was confirmed as an IE membrane protein using biochemical techniques and confocal microscopy. Aptamer-mediated affinity purification and tandem mass spectrometry enabled the identification of the likely aptamer target.

Discovery of a protein conserved between the major human malarias may have implications for vaccine development and validates the Serial-SELEX technique as a powerful tool for antigen discovery.

1152

ENHANCING ANTIBODY IMMUNOGENICITY OF TRANSMISSION-BLOCKING MALARIA VACCINES

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Transmission blocking malaria vaccines (TBVs) target *Plasmodium falciparum* sexual stages, aiming to block their further development within the mosquito. Different delivery systems, targeting Pfs25, a leading TBV candidate antigen have previously shown good transmission-blocking efficacy in pre-clinical models but these results have not translated in humans. One of the major challenges in translating pre-clinical efficacy of TBVs to humans has been the apparent need for exceptionally high antibody titres against known targets to achieve good transmission blocking activity. It has been shown that the IC50 (antibody concentration required to inhibit 50% of parasites) of anti-Pfs25 antibodies are 15.9, 4.2, 41.2, and 85.6 µg/mL for mouse, rabbit, monkey and humans respectively, and the differences among species are significant. To improve the Pfs25 antibody response we have fused Pfs25 to IMX313, a short DNA sequence which spontaneously forms a heptamer (PMID: 18474650). This increases the antibody levels by a log compared to Pfs25 alone after immunisation of mice with viral-vectors and improves transmission-blocking activity. We have set up the Pichia pastoris protein expression system as a viable GMP-compatible platform for production of monomeric Pfs25 and Pfs25-IMX313 fusion. Our results indicate that the heptamerisation improves the immunogenicity after protein-in-adjuvant immunisation as well. Immunogencity and transmission-blocking efficacy results will be presented.

1153

EVALUATION OF CANDIDATE *PLASMODIUM FALCIPARUM* VACCINE ANTIGENS USING THE RADIATION ATTENUATED SPOROZOITE VACCINE MODEL

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To aid development of a pre-erythrocytic subunit vaccine protecting against *Plasmodium falciparum* malaria, we sought to evaluate antigen-specific immune responses of human volunteers immunized with radiation attenuated *P. falciparum* sporozoites (RAS). Volunteers were exposed to RAS by the bites of irradiated, *P. falciparum* infected mosquitoes every four weeks for a total of seven immunization sessions. PBMCs and sera were acquired by leukapheresis following the final immunization. Subsequently, volunteers were challenged by mosquito-bites of *P. falciparum*-infected mosquitoes. A panel of 21 candidate pre-erythrocytic antigens was selected for evaluation. To first evaluate the development of antibodies targeting each candidate antigen, protein was produced using the wheat germ cell-free expression system (Cell Free Sciences) and serum reactivity was evaluated by Western blot. Of 21 candidate antigens evaluated, 20 antigens were recognized by the sera of one or more volunteers. To evaluate memory T cell reactivity to these candidate antigens, cultured ELISpot was performed. Briefly, PBMCs were stimulated with HLA-predicted peptides and/or pools of overlapping 15mer peptides for 12 days, washed, and restimulated for 18 hours. The number of interferon-gamma producing T cells was evaluated by ELISpot. Of the 21 candidate antigens screened, 15 were recognized by PBMCs of at least one volunteer and 6 were negative for all volunteers. We observed a trend toward increased breadth of antigens recognized by individuals who were protected from *P. falciparum* challenge compared to unprotected individuals, although this did not reach statistical significance. Antigens demonstrating reactivity among protected individuals have been prioritized for further evaluation and development as candidate vaccine antigens.

1154

GENERATION OF "FULLY HUMAN" MONOCLONAL ANTIBODIES TO *PLASMODIUM FALCIPARUM* FROM HUMAN-IMMUNE-SYSTEM HUMANIZED (DRAG) MICE

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Fully human monoclonal antibodies (mAb) are envisioned as a new therapeutic approach for neutralization of infectious agents/toxins, while devoid of side effects associated to the use of mouse, chimeric, or humanized antibodies. The current challenge for generation of fully human mAbs is the paucity of specific B cells in human blood, since antibody-secreting plasma cells reside in lymphoid organs and bone marrow. Approaches that use EBV-transformed human B cells impose difficulties for further development into clinical use, as EBV is a relevant human pathogen. We have generated humanized mice expressing HLA-DR4 molecules in a NOD.RagKO.IL2RgcKO background (DRAG mice). Upon infusion of HLA-DR-matched human hematopoietic stem cells the DRAG mice develop functional human B cells able to mount IgM and IgG antibody responses to malaria parasites. Herein we show the generation of a panel of fully human mAbs to *Plasmodium falciparum* sporozoites and blood stage parasites that inhibit parasite development. The humanized DRAG mouse model thus represents an easy, convenient, and powerful approach for generation of fully human therapeutic or phrophylactic (i.e., transmission- blocking) mAbs.

1155

IMMUNOGENICITY AND TRANSMISSION BLOCKING ACTIVITY OF PFS25-BASED VACCINE CANDIDATES IN RHESUS MONKEYS

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The Pfs25 protein is a lead candidate for transmission blocking vaccine (TBV) against malaria. The main challenge for recombinant Pfs25-based vaccine development is to increase functional immunogenicity of Pfs25. Previous studies demonstrated that conjugating *Pichia*-produced Pfs25 to protein carriers such as Outer Membrane Protein Complex (OMPC) from *N. meningitides* or detoxified ExoProtein A (EPA) of *P. aeruginosa* resulted in higher functional immune responses in animals. A Pfs25-EPA conjugate vaccine is currently being evaluated for safety, immunogenicity, and transmission blocking activity in a Phase 1 trial. Recombinant Pfs25 has also been expressed in plant tissues. The lead product on clinical development path is Pfs25-VLP, plant-produced Pfs25-AIMV coat protein fusion products self-assembled as a viral-like particle. A Phase 1 study on

Pfs25-VLP/Alhydrogel is scheduled to begin in 4th quarter 2013. A rhesus study is in progress to evaluate functional immunogenicity of 3 Pfs25-based products, Pfs25-EPA, Pfs25-OMPC and Pfs25-VLP, all formulated with aluminum adjuvants. The animals received 3 immunizations on D0, D56, and D112 or D168, similar to the dose-regimen designs in human trials. This design would allow evaluation whether rhesus can be used in preclinical studies to predict responses in humans. Antibody responses are measured by Pfs25-specific ELISA. Transmission blocking activity of vaccine-induced antibodies is measured by standard membrane feeding assays. The animals are being followed up to 12 months post the 3rd vaccination, for evaluation of response duration. Results from this study may facilitate candidate selection process for TBV product development.

1156

PLASMODIUM VIVAX ANTIGEN DISCOVERY BASED ON ALPHA HELICAL COILED-COIL PROTEIN MOTIFS

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Protein α-helical coiled-coil structures that elicit antibody responses able to block critical functions of medically important microorganisms represent a mean for vaccine development. By using bioinformatics algorithms of *Plasmodium falciparum* genome, functional α-helical coiled-coil structures were identified, chemical synthesis and multiple potential vaccine candidates are currently in pre-clinical and clinical development. We have used the same technology and approach to identify *P. vivax* antigens orthologous to *P. falciparum* antigens. A total of 40 proteins were evaluated for their antigenicity using sera samples of individuals from malaria endemic areas of Colombia; additionally, 8 of them were tested for their immunogenicity in BALB/c mice. Mouse antibody responses were determined by ELISA using as antigen the corresponding polypeptide and their reactivity with the native proteins was assessed by IFA test and western blot techniques using *P. vivax* blood stages. Analysis of these antigens reveals that such structures are highly antigenic and immunogenic, all fragments induced antibody response with titers variables from 104 to106 regarding to the antigen used. Additionally, the cellular response was evaluated through ELIspot assay for IFN-γ production in splenocytes from immunized mice. Six of the eight individuals induced IFN-γ production with media SFC from 60 to 300. Based on this results five protein fragments have been selected for immunogenicity and protective efficacy in Aotus monkeys. This approach combining α-helical coiled coil protein motifs and chemical synthesis could lead to the rapid identification and development of new malaria vaccine candidates.

1157

A CHIMERIC *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN VACCINE INDUCES HIGH TITERS OF PARASITE GROWTH INHIBITORY ANTIBODIES

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The C-terminal 19 kDa domain of *Plasmodium falciparum* merozoite surface protein 1 (*Pf*MSP1$_{19}$) is an established target of protective antibodies. However, clinical trials of *Pf*MSP1$_{42}$, a lead blood-stage vaccine candidate which contains the protective epitopes of *Pf*MSP1$_{19}$, revealed suboptimal immunogenicity and efficacy. Based on proof-of-concept studies in the *Plasmodium yoelii* murine model, we produced a chimeric vaccine antigen containing r*Pf*MSP1$_{19}$ fused to the N-terminus of *P. falciparum* merozoite surface protein 8 that lacked its low-complexity Asn/Asp-rich domain, r*Pf*MSP8 (ΔAsn/Asp). Immunization of mice with the chimeric r*Pf*MSP1/8 vaccine elicited strong T cell responses to conserved epitopes associated with the r*Pf*MSP8 (ΔAsn/Asp) fusion partner. While specific for *Pf*MSP8, this T cell response was adequate to provide help for the production of high titers of antibodies to both *Pf*MSP1$_{19}$ and r*Pf*MSP8 (ΔAsn/Asp) components. This occurred with formulations adjuvanted with either Quil A or with Montanide ISA 720 plus CpG ODN and was observed in both inbred and outbred strains of mice. *Pf*MSP1/8 induced antibodies were highly reactive with two major alleles of *Pf*MSP1$_{19}$ (FVO and 3D7). Of particular interest, immunization with *Pf*MSP1/8 elicited higher titers of *Pf*MSP1$_{19}$ specific antibodies when compared to a combined formulation of r*Pf*MSP1$_{42}$ and r*Pf*MSP8 (ΔAsn/Asp). As a measure of functionality, *Pf*MSP1/8-specific rabbit IgG was shown to potently inhibit the *in vitro* growth of blood-stage parasites of the FVO and 3D7 strains of *P. falciparum*. These data support the further testing and evaluation of this chimeric *Pf*MSP1/8 antigen as a component of a multivalent vaccine for *P. falciparum* malaria.

1158

CROSS-REACTIVITY OF ANTIBODIES FROM COLOMBIAN MEN AND CHILDREN EXPOSED TO *PLASMODIUM VIVAX* WITH THE *P. FALCIPARUM* VAR2CSA ANTIGEN

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During pregnancy, red blood cells infected with specific variants of *Plasmodium falciparum* sequester in the placenta through adhesion to chondroitin sulfate A (CSA). Sequestration is mediated by expression of PfEMP1 encoded by *var2csa*-type genes. Antibodies against these variants are associated with protection from maternal malaria. In most malaria-endemic settings, antibodies against VAR2CSA are infrequently observed in men, children, and non-pregnant women. Using enzyme-linked immunosorbent assay (ELISA), we detected antibodies against multiple constructs of VAR2CSA among Colombian men and children exposed only to *Plasmodium vivax*. We showed that DBL5e specific IgGs were present at significant levels among men (54%) and children (70%), as well as antibodies to DBL3X and ID1-ID2. Moreover, results showed that Colombian men and children had anti-VAR2CSA antibodies with high avidity, and a predominance of IgG1 and IgG3 subclasses. By competition ELISA assays, we demonstrated that purified IgG from men and naturally antibodies acquired by immune Beninese multigravidae shared common epitopes with VAR2CSA antibodies induced in immunized rabbits. These findings suggest that exposure to *P. vivax* can generate antibodies against VAR2CSA from *P. falciparum* in the general population in Colombia, which may alter the pathogenesis of malaria in regions where both species co-exist.

1159

DRAG MICE WITH A HUMAN-IMMUNE SYSTEM, DEVELOP HUMAN HEPATOCYTES AND ERYTHROCYTES AND SUSTAIN THE COMPLETE LIFE CYCLE OF *PLASMODIUM FALCIPARUM* MALARIA PARASITE

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Malaria is a deadly infectious disease affecting millions of people in the tropical countries across the world. Among the five species of *Plasmodium* parasites that affect humans, *P. falciparum* accounts for most morbidity and mortality associated to malaria. The lack of convenient animal models able to sustain liver- and blood-stage *P. falciparum* infection has hindered the understanding of disease pathogenesis and vaccine testing prior to clinical trials. We have previously showed that humanized DRAG (HLA-DR4.RagKO.IL2RgcKO.NOD) mice infused with hematopoietic stem cells from umbilical cord blood generate a functional human immune system and respond to vaccination. Herein, we show that the humanized DRAG mice also develop human hepatocytes and human erythrocytes and sustain blood-stage *parasitemia* upon challenge with *P. falciparum* sporozoites. DRAG mice thus represent the first small rodent model to support the complete life cycle of *P. falciparum*. The ability of DRAG mice to develop a functional human immune system and sustain *P. falciparum* infection provides a unique model to test the immunogenicity and protective efficacy of human malaria vaccine candidates.

1160

CSP AND AMA-1 ANTIGEN-SPECIFIC REFERENCE REAGENTS FOR THE STANDARDIZATION OF MALARIA VACCINE CELLULAR IMMUNOLOGIC ASSAYS

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Malaria causes significant mortality worldwide, and there is an urgent public health need for a vaccine. Several promising candidate vaccines to prevent *Plasmodium falciparum* are currently being evaluated in clinical trials around the world. Of these, the best vaccines will be down-selected based on favorable safety and tolerability profiles, superior immunogenicity and high protective efficacy. The down-selection process with regard to immunogenicity would be improved by the generation of standard reagents that could then be used as negative and positive controls across diverse assays for cell mediated immunity (CMI). Several barriers have existed to generating such reagents: peripheral blood mononuclear cells (PBMCs) from human research subjects are limited in quantity, antigen-specific cellular responses have generally been low, and until recently, there have been no candidate malaria vaccines that predictably induced strong CD8+ T cell responses in humans. The Malaria Department of the Naval Medical Research Center now has an opportunity to generate these reagents using the NMRC-M3V-AdPfCA Vaccine, a highly immunogenic adenovirus (serotype 5)-vectored vaccine encoding the Pf CSP (expressed in sporozoite and early liver stages) and PfAMA1 (expressed in sprozoite, liver and erythrocytic stages) antigens. In our experience, a single, low dose immunization with this vaccine (2x1010 particle units), delivered intramuscularly without adjuvant, induces brisk antigen-specific cellular immune responses (average IFN-γ secreting cells to CSP of 400-500 per million PBMC and to AMA1 of 900-1100 per million PBMC). For this reason, we are conducting a Phase 1 trial in which eligible subjects will receive a single administration of the Ad-PfCA vaccine with large quantities of PBMCs collected via leukapheresis pre- and post-immunization. We anticipate that this clinical trial will provide invaluable reagents to expedite the development and optimization of novel cell-mediated assays.

1161

A RETROSPECTIVE ANALYSIS OF THE ADVERSE EVENT DATA FROM THE PHASE 1 TRIAL *PLASMODIUM SP.* SPOROZOITES IMMUNIZATION OF HUMAN VOLUNTEERS

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In 2002-2003, we immunized research subjects with radiation-attenuated *Plasmodium falciparum* sporozoites (RAS) via mosquito bite, a model for vaccination against malaria that historically has induced high grade (>90%) protection against controlled human malaria infection (CHMI) following > 1000 immunizing bites. Leukapheresis was conducted before and after RAS immunization to collect large numbers of peripheral blood mononuclear cells (PBMC) to characterize protective immune responses and identify protective antigens for malaria vaccine development. Volunteers were placed in two main groups: immunization with RAS, and mock immunization with uninfected mosquitoes. We herein report the safety data collected during the trial. 57 volunteers were screened, 41 enrolled, and 27 received at least one immunization session. Of the 27, source documentation for 22 was available for retrospective analysis. The results revealed that immunization via mosquito bite was generally well tolerated, but could also lead to significant adverse events (AEs). Local AEs were consistent with reactions to mosquito bites in the wild, consisting of erythema, papules, swelling, and induration; however, two individuals, one true and one mock immunized, developed generalized swelling of the forearm (large local reactions) and were withdrawn from further participation. Systemic AEs were generally rare and mild, consisting of headache, myalgias, nausea, and low grade fever; however, two subjects experienced the abrupt onset of a symptom complex characterized by fever, malaise, myalgia, nausea and rigors consistent with a form of serum sickness secondary to a concentration of pre-formed antibody reacting with mosquito salivary antigens. Overall, our retrospective analysis of *Pf*RAS immunization via mosquito bite revealed that, although the method is generally safe and well tolerated, large local reactions to the mosquito bites and systemic adverse reactions of moderate severity may occur, and may relate more to mosquito salivary antigens than to the malaria sporozoites.

1162

PLASMODIUM VIVAX SPOROZOITE CHALLENGE METHOD FOR MALARIA VACCINE AND DRUG CLINICAL DEVELOPMENT

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Important efforts have been performed towards the development of malaria vaccines including *Plasmodium vivax*. We have established a safe, reliable, and reproducible sporozoite infectious challenge method in Colombia. Two infection trials have been conducted in naïve human volunteers that have been exposed, in a first challenge to the bite to 2-4, 5-7 and 8-10 mosquitoes of *Anopheles albimanus* mosquitoes infected from one *P. vivax* donor and in a second challenge, volunteers where exposed to the bites of 2-4 mosquitoes infected from different donors. Pre-patent periods were similar in both trials, between 9-13 days (mean 10.6) in the first and 9-16 days (mean 12) in the second trial as determined by Thick Blood Smear. All volunteers were closely followed for clinical appearance of malaria and where treated immediately *parasitemia* became patent. All participants successfully recovered from malaria after treatment, with no serious adverse events. In preparation for Phase IIa/

IIb vaccine trials, a third trial has been designed to determine differences between naïve individuals and volunteers having been previously in contact with malaria (pre-immune). We will compare the length of pre-patent period, clinical manifestations and immune responses of both groups. A total of 19 volunteers, 7 naïve from a non-endemic area and 12 pre-immune from endemic area were enrolled. Volunteers are men (n=12) and female (n=7) with 18-45 y/age. Naïve volunteers have been confirmed to be negative for malaria by ELISA and IFAT, whereas pre-immune have displayed reactivity against PvMSP-1 and PvCS (1:200 titers both) by ELISA and *P. vivax* blood stages by IFAT (1:40 to 1:160 titers). Sera and cells of all volunteers will be collected before and during the trial to determine antibody and cell mediated responses. We would assess the induction of antibody responses in naïve volunteers and the potential boosting effect of infection in pre-immune volunteers. Additionally, cytokine levels, as well as both B cell and monocyte subpopulation profiles will be evaluated. Results of this ongoing trial will be presented.The described method is critical for phase IIa/b vaccine trials and is contributing to accelerate the clinical development of *P. vivax* vaccines and would be valuable for testing of antimalarial drugs targeting liver parasite forms.

1163

URINARY TRACT INFECTION: PREVALENCE, PATHOGENS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN AMONG FEBRILE CHILDREN AT MWANANYAMALA HOSPITAL, TANZANIA

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Urinary tract infection (UTI) is a common and important cause of morbidity in the pediatric population in developing countries. Prevalence rates of UTI in children ranges from 3.3% in the United States to 39.7% in Northwestern Tanzania.Many uropathogens are developing resistance to antibiotics recommended by WHO to treat UTI.The magnitude, etiology and antimicrobial susceptibility of UTI in Tanzanian febrile children are not well defined. All published studies to date on the prevalence and etiologies of UTI in febrile children have not included a control group which is necessary to investigate false positives due to poor collection procedures. The aim of the study was to determine the prevalence of UTI, pathogens and antimicrobial susceptibility in febrile children aged 2-5 years at Mwananyamala District Hospital (MDH). This was a cross-sectional descriptive hospital-based study. Febrile and afebrile children were consecutively recruited from the paediatric outpatient clinics at MDH. Urine culture and sensitivity testing was performed for febrile and afebrile children who had positive urine dipstick tests for nitrates and leukocyte esterase. A total of 556 children were enrolled into the study.Of these 370(66.5%) were febrile and 186(33.5%) were afebrile.Prevalence of UTI among febrile children was 7.8%. Females had higher prevalence than males, however, the difference was not statistically significant(p=0.418). Prevalence of UTI among afebrile children was 5.9%. Febrile children were noted to have higher prevalence of UTI than afebrile children, though the difference was not statistically significant (p=0.729). *Escherichia coli* (38%) was the most commonly isolated organism followed by *Klebsiella* (21%). Resistance rates of the isolated bacteria to Cotrimoxazole, Erythromycin, and Amoxillin were 100%,89.7% and 86.2% respectively. Gentamycin and Ceftriaxone had slightly lower resistance rates of 48% and 57% respectively while Amikacin and Ciprofloxacin had least resistance rates of 0% and 6.9%respectively. In conclusion prevalence of UTI among febrile children aged 2-5 is 7.8% and the commonest bacteria isolated were *E. coli* and *Klebsiella* which showed a high resistance to Amoxillin, Cotrimoxazole and Erythromycin. Further long term studies should be conducted among this age group so as make changes to the current standard treatment for UTI.

1164

STUDIES OF *ORIENTIA TSUTSUGAMUSHI* PERSISTENCE IN NEWLY DEVELOPED ANIMAL MODELS

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Orientia tsutsugamushi is a gram-negative obligately intracellular coccobacillus. It is the causative agent of scrub typhus, a serious public health problem in Asia and the islands of the Pacific and Indian oceans. It can cause severe multiorgan failure with a mortality rate of 7-15% without appropriate treatment. The antigenic heterogeneity of *Orientia* plays a role in scrub typhus reinfection. As a neglected disease, there are still gaps in understanding how *O. tsutsugamushi* invades, disseminates, and interacts within the host. We have developed new mouse models for *Orientia* infection, which better mimic the pathology and immunology in human patients. The histopathology and cytokines from relevant organs such as lung and liver confirmed that our intravenous (i.v.) and intradermal (i.d.) mouse models are valid for the study of scrub typhus. Real-time PCR and immunofluorescence staining demonstrated that kidney was the main target organ with 34,250 to 270, 000 copies of *O. tsutsugamushi* per kidney during the persistent infection after i.v. and i.d. inoculation. Seventy five percent fewer copies of *O. tsutsugamushi* were recovered from the corresponding lung samples, the main target during acute *Orientia* infection, than kidney. These bacteria persistent in the mammalian host were viable and virulent. Histologic studies showed lesions (vasculitis, and interstitial nephritis) in the renal tissue. We also immunosuppressed *Orientia* infected mice with cyclophosphamide or radiation 82 days post infection. The immunosuppressive treatments did not result in clinical recrudescence of scrub typhus. All kidneys, lungs, spleens, livers and lymph nodes collected from those immunosuppressed and infected mice contained virulent *O. tsutsugamushi* that were infectious for naive mice. We also found changes of host immune cells and their cytokines, but further studies are required. We are currently investigating the mechanisms behind these phenomena, and we hypothesize that *Orientia* finds and induces loopholes in host immunity to avoid being cleared.

1165

LEPROSY ELIMINATION IN BANGLADESH: A NEGLECTED DISEASE REVISITED IN 2012

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In 2013, Leprosy remained as a neglected disease in Bangladesh. Though the WHO declared elimination goal was achieved in 1998, yet the national Leprosy elimination Programme (NLEP) statistics started to show stagnancy in prevalence, new case detection and variations in other parameters. This is more noticed in the north and north eastern part of the country, the area chronically suffers from poverty, seasonal famine and lack of common nutrients. Data from the national programme (NLEP) and its implementing partners are analyzed. Two workshops aiming at formulating elimination strategies for 2011-16 and 2015 were organized in 2012 and 2013. Issues and concerns from those meetings are incorporated. NLEP is detecting around 4-5 thousands new cases each year since 2006. However, out of 64 districts, 13 endemic areas having 25 % of the total population, contributing to 59% of the all the cases detected in 2012. Fifty percent of them were MB cases, 57% of the smear positive, 68% of the ≤ 15 years cases, and 58% of the visible disabilities cases belonged to these 13 areas. Loss of focus in elimination activities, less fund allocation, rapid burn out of skilled personnel at field level, and huge lack of awareness about the

disease are major concerns. Complacency in elimination activities and lack of any interest in leprosy elimination created an emergent threat of this neglected disease affecting mostly the poor people of the society.

1166

MODULATION OF IMMUNIZATION VIA CHOLINERGIC NERVOUS SYSTEM USING HI-6

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HI-6 is a compound known is some sources as asoxime. It is used as an antidote to nerve agents. When HI-6 administered to the poisoned one, it causes return of acetylcholinesterase (AChE) activity. In recent works, we proved that HI-6 acts as an antagonist to acetylcholine receptors (AChR) including the nicotinic receptor, alpha 7 nAChR which is involved in regulating the immune response through macrophages. The here presented experiment reports the efficacy of HI-6 to regulate the immune response. Laboratory BALB/c mice received HI-6 and/or keyhole limpet hemocyanin (KLH) as an antigen. Controls received saline or a combination of Freund´s complete adjuvant and KLH. Antibody production was investigated after either 21 or 65 days when either single or repeated dose of antigen was applied. We confirmed that HI-6 significantly improved vaccination efficacy when KLH was given in a dose of 1 mg/kg. The effect was dose dependent: repeated HI-6 produced no on further improvement of the vaccination. A combination of HI-6 and KLH produced a vaccination of almost the same efficacy as that for Freund´s complete adjuvant. The findings point at the suitability of HI-6 for improving vaccination efficacy at the level of immunity regulation by the nervous system.

1167

STAPHYLOCOCCUS AUREUS ADHESION-GENES EXPRESSION IN AN ORAL EPITHELIUM IN VITRO MODEL

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Staphylococcus aureus is an important nosocomial pathogen able to produce a great number of extracellular virulence factors and molecules associated to the cell wall, including several adhesins, such as the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). The aim of this work was to determine the expression of 12 adhesins in *Staphylococcus aureus* strains, isolated from catheters of hemodialysis patients, in an oral epithelium *in vitro* model. *S. aureus* was identified by manitol and coagulase biochemical tests, and by rRNA, *nuc* and *spa* (x region) PCR amplification. *S. aureus* strains were also investigated by PFGE (pulsed field gel electrophoresis) by *SmaI* DNA digestion and electrophoresis in CHEF MAPPER (Bio-Rad). After *S. aureus* oral epithelium infection, *S. aureus* RNA was extracted and subjected to reverse transcription and real time PCR of adhesion genes using Qiagen comercial kits. All *S. aureus* strains (n=21) expressed *bbp, clfb*, *cna,* and *sdrC* genes*;* 95.2% (n=20) *sdrD;* 90.4% (n=19) *map/eap;* 85.7% (n=18) *ebps*; 76.1% (n=16) *sdrE;* 47.6 (n=10) *fnbA, fnbB,* and *spa;* 28.5% (n=6) *clfA.* Results showed that *S. aureus strains* expressed different combinations of MSCRAMMs´s family genes during oral epithelium infection. Analysis of DNA restriction fragments by PFGE revealed that all *S. aureus* strains are different, suggesting that catheter contamination is not due to the hospital personnel manipulating hemodialysis equipment.

1168

DIFFERENTIAL GROWTH OF *RICKETTSIA FELIS* STRAINS RF2125 AND URRWXCAL2 IN TWO CELL LINES

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The importance of *Rickettsia* has increased in the past couple of decadesa, especially as intracellular bacteria responsible for emerging zoonotic diseases. *Rickettsia* felis is considered one of these emerging pathogens in Latin America, and it has been associated with human disease in Brazil, Mexico, and Colombia. Recently in Costa Rica, two different strains of *Rickettsia* felis from fleas have been isolated in cell culture. The aim of this study was to determine the growth characteristics of strains RF2125 and URRWXCal2 in Vero and C6/36 cell lines. Two isolates of R. felis strain RF2125 and one of strain URRWXCal2 all from Ctenocephalides felis of Costa Rica were inoculated at 1.38x106 bacteria/ml in bottles with confluent monolayers of C6/36 cells in RPMI with 2.5% fetal calf serum and Vero cells in MEM with 4% newborn calf serum. Growth was evaluated in both cell lines with and without a 2% triptose supplement and at 28 °C and 32 °C. Bacterial growth was evaluated every week for a period of one month, using a semiquantitative scale of 1+ to 4+ (+ to ++++) according to the number of bacteria per cell observed with a Giménez stain. Both isolates of R. felis RF2125 grew well (+++ to ++++) in Vero cells with and without triptose at both temperatures, although growth was slower in one of the bottles without triptose at 32 °C. Growth of R. felis RF2125 isolates was minimal (+) after 4 weeks in C6/36 cells in medium with and without triptose and at both temperatures. *Rickettsia* felis isolate URRWXCal2 grew moderately (+++) in C6/36 cells at a 28 °C with and without triptose, as well as at 32 °C with triptose. Its growth was reduced (++) at 32 °C without triptose, and it only slightly grew (+) in Vero cells at 28 °C with triptose. Both isolates of R. felis RF2125 exhibited similar growing characteristics, with better growth in Vero cells. The isolate of R. felis URRWXCal2 was completely different as it grew better in C6/36 cells than in Vero cells. A triptose supplement in the medium favored growth of both R. felis strains in cell culture. Results show that there are differences at the metabolic level and/or specific receptors that should be further evaluated.

1169

ASSESSMENT AND COMPARISON OF THE HUMAN IMMUNOLOGICAL RESPONSE TO ANTHRAX INFECTION AND VACCINES

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Understanding immune responses to anthrax remains a priority in biodefense countermeasure development. Currently, three different anthrax vaccines are employed for human protection. The US Anthrax Vaccine Adsorbed (AVA, BioThrax) represents a cell free preparation of protective antigen (PA). Live attenuated anthrax vaccine (LAAV) is licensed for human use in former Soviet Union countries. Wecompared immune responses elicited by different vaccines with those in patients diagnosed with cutaneous anthrax.Sixty three clinical serum samples were evaluated

from subjects with either LAAV (n=16) or AVA (n=11) vaccinations, cutaneous anthrax patients (n=28) and naïve controls (n=8). Samples were tested in quantitative anti-PA, endpoint anti-LF and anti-EF IgG ELISAs, and toxin neutralization assay (TNA). Antibody titer data was transformed to remove unequal variance and analyzed by multivariate linear modelling. Significantly raised anti-PA antibody titers, when compared to controls (geometric mean (GM) = 0.23 µg/mL), were found in the anthrax patients (GM = 3.14 µg/mL, P=0.001) and the AVA recipients (GM = 35.63 µg/mL, P<0.001) and weakly higher in LAAV vaccinees (GM = 1.78 µg/mL, P=0.096). Strongest anti PA antibody responses were observed in the AVA vaccinees with levels significantly higher than those in other groups (P<0.002,). Anthrax patients demonstrated significantly raised anti-LF responses (P<0.001) when compared to controls. No significantly raised anti-EF responses were observed (P>0.10). The TNA ED50 titers were compared by Dunn's multiple comparisons. We found that TNA ED50 titers were significantly raised, when compared to controls (0% responding), in the AVA (72.7% responding, P=0.012) vaccinated group, but not the anthrax patients (39.3% responding, P=1.0) or the LAAV vaccinees (18.8% responding, P=1.0). We found that none of the factors, time elapsed since exposure anthrax antigen, gender and subject age effected antibody titer with the exception of number of doses effecting anti PA antibody titres (P=0.001), used as a covariate in multivariate analysis of antibody data. The vaccines included in this study are regarded as protective and the variations in human response to these vaccines observed here are a demonstration that correlates of protection need to be thought out carefully.

1170

LEPROSY AMONG THE FOREIGN-BORN IN THE UNITED STATES, 2000-2010

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Leprosy (Hansen's disease, HD) is a neglected tropical disease and, although rare, may cause severe disability. Over the last 30 years, the HD burden has declined worldwide, in large part due to implementation of free short-course multiple-drug therapy. Still, HD remains endemic in many less-developed countries. In the United States (US), HD is a rare disease mostly associated with immigrants from endemic areas. We reviewed surveillance data on HD in the US, with a focus on foreign-born populations. Data were obtained from the National Hansen's Disease Registry for the period 2000-2010. We used chi-square and t-tests to compare characteristics of foreign-born and US-born cases. A total of 1,439 HD cases were diagnosed during 2000-2010, an average of 130 cases per year. Persons with cases resided in 49 states, the top five states being Texas (15%), California (14%), Hawaii (10%), Florida (8%), and Louisiana (7%). Seventy-three percent of cases were in foreign-born persons, for whom the top countries of birth were Mexico (20%), Micronesia and Trust Territories (18%), Brazil (12%), the Philippines (11%), and India (11%). Rates of HD among the foreign-born peaked in 2003 (0.33 per 100,000) and declined to 0.21 per 100,000 by 2010. Rates for the US-born remained stable (0.01 to 0.02 per 100,000) during the study period; in 2010 rates among the foreign-born were 21 times higher than among the US-born. Seventy percent of cases were in males. The median age at diagnosis was significantly lower (p<0.05) for the foreign-born (35 years) than the US-born (56 years). The median time from arrival in the US to diagnosis was 6.3 years. Leprosy remains a health disparity for the foreign-born in the US. Persons from endemic countries need to be targeted for disease education, case-finding, and case-management interventions. Further research is needed on the epidemiology of HD in the US, and specifically on the reasons for diagnosis of HD years after arrival in the US.

1171

AR-12: A BROAD SPECTRUM HOST CELL-DIRECTED INHIBITOR OF INTRACELLULAR PATHOGENS

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AR-12 is an IND approved, COX-2 inhibitor derivative that affects host cells primarily by up-regulating autophagy. In vitro, AR-12 has shown broad-spectrum efficacy against several bacteria and parasites including *Salmonella typhimurium* and *Francisella tularensis*. Although for some pathogens, the drug's effect is observed directly against the pathogen, AR-12 shows its strongest promise as a host-targeted therapeutic, which makes it ideal for treatment of multidrug resistant agents. In vitro analysis of AR-12 for *S. typhimurium* -infected macrophages displayed a MIC of 0.5 µM; however, 5 mg AR-12/kg in BALB/c mice did not result in significant increase in overall mouse survival with S. Typhimuriumm infection. Reduced *in vivo* efficacy is likely due to drug dosing limits because of poor drug solubility and biodistribution. To overcome solubility issues, we have encapsulated AR-12 in acetalated dextran (Ac-DEX) nanoparticles (NPs) that passively target phagocytes (the preferred host cell of these bacteria), while non-phagocytic cells are not capable of internalizing the particles. By passively targeting the phagocytes, we can achieve targeted delivery of the compound directly inside these cells, thus limiting drug side effects and perhaps enhancing the action of the drug, compared to unencapsulated parenteral administration. Ac-DEX is an acid sensitive polymer with tunable release kinetics that will degrade and release drug in the low pH environment of phagocytes' phagosome. This biodegradable polymer is superior to other commonly used biomaterials (e.g. poly lactic-co-glycoic acid (PLGA)) because it degrades into pH neutral dextran and low levels of ethanol and acetone. We have demonstrated that AR-12 encapsulation into Ac-DEX NPs can significantly reduce drug-induced cytotoxicity in human monocyte-derived macrophages (MDMs) and that higher drug concentrations can be achieved intracellularly. Furthermore, we have evaluated the encapsulated drug against *Mycobacterium tuberculosis*, *S. typhimurium* and *F. tularensis* in host MDMs. These studies will help to develop and characterize a new broad-spectrum antibiotic and delivery platform that targets the host and inhibits bacterial survival by increased co-localization of intracellular bacteria with autophagosomes. Such a platform would help eliminate the pathogen and limit the emergence of multidrug resistance.

1172

FREQUENCY OF GES β-LACTAMASES IN *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES *IN* LIMA, PERÚ

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GES β-lactamases are a group of enzymes with both extended-spectrum beta-lactamase (ESBL) and weak carbapenemase hydrolysis activity. The GES β-lactamases play an important role in the spread of antibiotic resistance and complicate treatment choices for *Pseudomonas aeruginosa* infections. GES β-lactamases in multidrug resistant *P. aeruginosa* have been widely described worldwide, however scarce information is available in Latin American countries. We tested 149 *P. aeruginosa* clinical isolates obtained in 5 hospitals from Lima during July 2010 to July 2012. Resistance patterns were determined by the disk diffusion test and interpreted according to CLSI guidelines. Multidrug resistant was defined as the resistance to at least two of the following antibiotics families: β-lactams, quinolones, or aminoglycosides. To screen for GES β-lactamase activity, the double disk diffusion phenotypic test using ceftazidime and imipenem disks was performed and the genes encoding VEB, PER, TEM, SHV and OXA-1-like β-lactamases were detected by PCR. We additionally performed PCR for the detection of GES β-lactamases genes and determined the total frequency of GES β-lactamases among the *P.*

aeruginosa isolates to be 42% (63/149) with a 7% (10/149) concordance with the isolates phenotype. The frequency of genes encoding TEM and OXA-1-like β-lactamases was 7% and 13%, respectively. Genes encoding VEB, PER and SHV were not detected in these clinical isolates. Among the GES β-lactamases producers, we found that 13% (8/63) of these isolates also had genes encoding TEM β-lactamases and 8% (5/63) had genes encoding OXA-1-like β-lactamases. In addition, 87% of the *P. aeruginosa* isolates were multidrug resistant. There was a strong association between the presence of GES β-lactamases genes and those isolates which were multidrug resistant ($p<0.001$). In conclusion, 35% of the GES-positive *P. aeruginosa* isolates would have been missed by conventional phenotypic methods and supports the use of molecular methods to more reliably detect antibiotic resistance within the hospital.

1173

POINT-OF-CARE DIAGNOSIS FOR THE EMERGING SKIN DISEASE, *BURULI* ULCER: AN ANTIGEN CAPTURE APPROACH

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Buruli ulcer (BU) is a chronic and destructive skin disease caused by infection with *Mycobacterium ulcerans*. Since the mode of transmission is still not entirely explored and there is no vaccine to prevent the disease, the current strategy to control BU relies on early case detection and antibiotic treatment. In remote areas of West and Central Africa, which are most affected by the disease, the diagnosis of BU is primarily based on clinical findings. However, the differential diagnosis for non-specific early stages of BU is broad. A specific and sensitive diagnostic test, which can be performed without expensive equipment and qualified personnel, could greatly support the diagnosis of M. ulcerans infection at field sites. While we have shown in our previous work that antigenic cross-reactivity between different mycobacterial species complicates the development of a specific serological test, we are now focusing on a direct detection of M. ulcerans antigens within the BU lesions. For this purpose, we generated high affinity antibodies against a set of highly abundant M. ulcerans protein antigens and evaluated their suitability as key reagents in ELISA-based antigen capture assays. Analyses of partial swab samples taken from BU lesions revealed for one of the antigens a sensitivity rate comparable to that of microscopic detection of acid fast bacilli in smears from clinical specimens. Systematic optimization of ELISA parameters further improved the sensitivity of antigen detection. We are now testing full BU swab samples in order to compare the performance of the developed assay with real-time PCR analysis, which is the current gold standard for BU diagnosis in reference laboratories. In addition, we are analyzing the potential application of the developed immunological reagents in other field-compatible assay formats.

1174

DETECTION OF MUTATIONS IN GYRA GENE OF FLUOROQUINOLONE-RESISTANT *NEISSERIA GONORRHOEAE FROM* SWABS AND URINE SAMPLES

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Gonorrhea is the second most commonly reported bacterial sexually transmitted infections (STI), and a public health problem of great importance. Approximately 95% of heterosexual men are symptomatic, while more than 60% of women may be asymptomatic carriers. Among men who have sex with men (MSM), pharyngeal and rectal infections are common and mostly asymptomatic. Increasing antibiotic resistance presents a significant problem for gonorrhea treatment and it is essential to maintain continuous monitoring of strains for resistance to first-line antibiotics such as fluoroquinolones. In this study 1140 samples (pharyngeal and rectal swabs and urine) were tested by the GenProbe APTIMA CT/GC Transcription Mediated Amplification (TMA) assay which yielded 59 *Neisseria gonorrhoeae* positive-samples. Of these 11 of the 59 (18.6%) samples were found resistant to fluoroquinolones (QRNG) by Real Time PCR. Four (6.8%) were heterozygous, 14 (23.7%) had wild-type *N. gonorrhoeae* and 34 (57.6%) did not amplify. QRNG were sequenced and analyzed and identified one (9.1%) with single mutation S91I, two (18.1%) with single mutation S91F, two (18.1%) with double mutations S91F and D95A and six (54.6%) with double mutations S91F and D95G in the gyrA gene.In conclusion, detecting QRNG from invasive and noninvasive samples is important for monitoring antimicrobial resistance. In the last decade, fluoroquinolones have been used as antibiotics to treat gonorrhea but in recent years reports of quinolone-resistant strains have appeared in several countries. The presence of QRNG with double mutation (S91 and D95) indicates a high resistance to quinolones and suggests they should no longer be used as first-line therapy in Peru.

1175

IMPLEMENTATION OF AUTHORITY GUIDELINES AND INNOVATION WITH EMPHASIS ON PREVENTION OF HEALTHCARE-ASSOCIATED SYMPTOMATIC CATHETER-ASSOCIATED URINARY CATHETER INFECTIONS (CAUTIS)

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The 600-bed hospital embraced the Centers for Medicare and Medicaid Services (CMS) challenge to address healthcare-associated infections (HAIs) as "never-events" by focusing on improving team communication, patient safety and quality through interventions to decrease the rates of symptomatic healthcare catheter-associated urinary catheter infections (CAUTIs). The Hospital Board of Directors established a goal to reduce the count of symptomatic catheter-associated UTIs by 10%, from a baseline (Jan 2008-Dec 2008) of 57 symptomatic CAUTIs to 51 symptomatic CAUTIs in Fiscal Year/FY 2010 (July 2009-June 2010). A multidisciplinary project management team, directed by the Infection Prevention and Control (IPC) team, included Clinical Nurse Specialists/CNS; front line staff; the IPC Committee; Clinical Analysts; Medical Staff; Chief Medical Officer; and Clinical Educators) used to implement the initiative. A urinary catheter bundle was implemented by: setting clear expectations for indications for indwelling urinary catheter use (an indwelling catheter policy which included criteria for catheter use); catheter insertion using aseptic technique and sterile equipment; use of small size catheters; catheter maintenance; hand hygiene; documentation of catheter placement); daily assessment of the continued need for indwelling urinary catheter by a multidisciplinary team (bedside Nurses, CNS, IPC and Case Management); the use of a bladder scanner before catheter placement; the use of intermittent catheterization when indicated and the promotion of education of direct care givers in clinical areas when CAUTIs occurred. The hospital realized a 63% reduction in symptomatic CAUTIs from MRSA cases of colonization and infection between the 2008 period (Jan 1, 2008-Dec 31, 2008) and 2009 period (Jan 1, 2009-Dec 31, 2009). The fiscal year-to-date (through March 2010) rate was 17 cases of CAUTIs, which was well below the target of 51 cases for the FY (which ended in June 30, 2010).

1176

PATHOGENESIS AND TREATMENT OF *BURULI* ULCER - A HISTOPATHOLOGICAL PERSPECTIVE

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Histopathological analysis is a valuable tool to diagnose diseases, to characterize pathomechanisms and to monitor response to treatment. For a range of diseases histopathology is therefore part of the standard diagnostic protocol and treatment may be guided by the results of the analysis. Leprosy is one example, where the classification based on the presence of a set of histopathological features supports the choice of an adjusted treatment regimen. Within the framework of several clinical studies we have performed histopathological and immunohistochemical studies with tissue samples from *Buruli* ulcer patients before, during and after treatment. These analyses have shown that *Mycobacterium ulcerans* is focally distributed in the lesions, with bacteria being mostly present in the deep subcutaneous fat layers and only rarely in the dermal layer of the skin. Immunohistochemical studies for sets of markers demonstrated a remarkable diversity in both the presentation of untreated lesions and in the response to treatment. In combination with clinical features, histopathology may therefore help to develop a more differentiated classification of *Buruli* ulcer lesions. Furthermore immunohistochemical analyses allow monitoring the transition from the inflammatory to the healing phase during treatment of *Buruli* ulcer. Results can give insight into reasons for retarded healing and may support clinical decision making.

1177

STUDYING MYCOLACTONE-TOXIN ASSOCIATED GENE EXPRESSION DURING *BURULI* ULCER INFECTION OF THE HUMAN HOST

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Mycobacterium ulcerans (MU) is the causative agent of *Buruli* ulcer (BU), a dramatic yet neglected disease found predominantly in tropical riverine regions of West and Central Africa. BU is a chronic destructive infection of subcutaneous tissue where lesions progress from a subcutaneous nodule to an ulcer of increasing size. Despite the extensive tissue damage, little or no inflammatory response occurs at this stage, and lesions are often painless. This unique pathology is attributed largely to the MU-secreted mycolactone, an toxin with cytotoxic, immunosuppressive, analgesic, and potent necrotizing activity, resulting in extensive ulcerative lesions. We hypothesized that higher levels of mycolactone associated gene expression in tissue samples from BU lesions correlate with larger and more advanced BU and predict an increased need for early surgery and worse patient outcome. To investigate this we first optimized RNA stabilisation from mammalian tissues to ensure immediate preservation of both host- and mycobacterial RNA's after surgical excision of affected patient tissues. By dissecting tissues to pieces <1mm immediately after collection and mixing these pieces within 5 minutes with the RNA stabilizer guanidine thiocyanate (GTC), we obtained non-degraded high quality mycobacterial RNA after phenol-chloroform extraction. We then started sampling ulcerative lesions from BU suspected patients, before initiation of antibiotic therapy, and after informed consent was given. Patients were recruited in Allada (Benin) through active and passive case-finding activities in the framework of a parent study to the differential diagnosis of BU. We then determined expression of MU mycolactone associated genes during human infection with quantitative reverse transcription-PCR (qRT-PCR). Relative quantification was performed with different reference genes which allowed accurate internal normalization of qRT-PCR data by geometric averaging of multiple internal control genes. We are currently in the process of assessing the relationship between mycolactone expression and BU lesion stage/patient outcome.

1178

HOSPITAL ACQUIRED INFECTIONS WITHIN THE AMAZON OF PERU

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Increasing bacterial antimicrobial resistance has been observed worldwide and in particular has complicated hospital acquired infections. Although, this problem is well described within developed countries, little is known about nosocomial infections within the Amazon region of South America. In this study, isolates from suspected nosocomial infections were collected from 2 major public hospitals in Iquitos, Peru (Hospital Regional Loreto and Hospital Apoyo) along with pertinent clinical information from June 2011 through April 2013. Over this time period, 381 infections were cultured (229 from Hospital Apoyo and 152 from Hospital Regional) with the most frequent categorized infections being bloodstream infections (37.4%), surgical site infections (25.7%), urinary tract infections (21.7%), pneumonia (19.9%) and skin and soft tissue infections (10.5%). We were able to recover 255 isolates (172 from Hospital Regional and 83 from Hospital Apoyo) to include 63 *Escherichia coli*, 42 *Klebsiella spp.* (17 *K. pneumoniae*), 36 *Pseudomonas aeruginosa*, 31 *Acinetobacter spp.*, 30 *Staphylococcus aureus*, and 53 other. For *P. aeruginosa*, 19% was resistant to imipenem with 45% resistant to ciprofloxacin, however only 8% was resistant to cefepime. Forty-five percent of *S. aureus* was classified as methicillin resistant with only 8% resistance to sulfamethoxazole/trimethoprim. Acinetobacter isolates showed 28% resistance toward imipenem. *Klebsiella spp.* and *E. coli* isolates were found to be multidrug resistant with >80% resistance toward ceftriaxone and sulfamethozazole/trimethoprim and >60% resistance to ciprofloxacin and gentamicin but with almost no resistance to imipenem. In conclusion, multi-drug resistant bacteria from the *Enterobacteriaceae* family predominately complicate hospital acquired infection in Iquitos, Peru with imipenem remaining a reliable treatment for Gram negative infections. However, infection control measures should be employed to prevent further development of antimicrobial resistance within the hospital.

1179

MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA* OUTBREAK IN A HOSPITAL IN LIMA, PERU

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As healthcare facilities improve in developing countries, nosocomial infections are of increasing concern. Rapid laboratory based detection allows tracking of the spread of multidrug-resistant pathogens and guides transmissions control measures in the hospital settings. The aim of this investigation was to determine if a series of *Pseudomonas aeruginosa* infections in a military hospital in Lima, Peru were transmitted nosocomially. Swab samples from 4 nosocomial infections were collected by the infection control program at one military hospital in Lima. Samples were processed using conventional bacterial culture and antimicrobial susceptibility testing. The 4 isolated *P. aeruginosa* strains were sub-cultured and subjected to rep-PCR amplification and fingerprinting using the Biomerieux Diversilab bacterial kit. Genotyping results from the DiversiLab determined the level of epidemiological concordance between the strains. The 4 patients ranged in age from 50 to 72 and had spent 15-240 days within the hospital prior to infection. 3 samples were taken from

patients with nosocomial infections related to surgical procedures and the remaining sample was taken from a groin of patient with complicated skin infection. No more samples were taken seeking source of transmission. Of the 4 pseudomona infections, one patient died, two patients were treated with only supportive care with complete remission of the infection, and the remaining patient had colonization. Sub-cultures isolated multidrug-resistant *Pseudomona aeruginosa* from each patient resistant to amikacin, cefepime, ceftazidime, ciprofloxacin, meropenem. All isolates were non susceptible to aztreonam. The Diversilab system determined that all isolates were greater than 96% related, strongly suggesting nosocomial transmission among these patients capable of causing different clinical outcomes, including dead. In this case, fingerprinting using the Diversilab system proved to be a valuable identification tool in the investigation of a nosocomial outbreak of *Pseudomona aeruginosa.*

1180

TRANSMISSION OF BRUCELLOSIS IN SLAUGTHERHOUSE WORKERS AND MILKERS IN THE MUNICIPALITY OF MONTERIA, CORDOBA, COLOMBIA

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Brucellosis is a bacterial zoonosis, considered occupational disease. In Colombia there are few studies of transmission to humans with temporally and geographically isolated data. The people risk group is composed by slaughterhouse workers, milkers, veterinarians and bacteriologists who process the samples in the laboratory. Traditionally, the diagnosis has been made by Rose of Bengal and confirmed by competitive ELISA or isolation of pathogen. The Rose of Bengal test is not sufficiently specific (54%), the competitive ELISA is very expensive and pathogen isolation is time-consuming and delayed. Establish the prevalence of brucellosis in slaughterhouse workers and milkers in the municipality of Monteria, Cordoba, Colombia. Blood samples from 162 workers were analyzed by Rose of Bengal and competitive ELISA assay. A survey to determine the degree of knowledge and attitudes that have volunteers about the problem was applied. It was found that 9 samples were positive by Rose of Bengal, these sample were confirmed by competitive ELISA. Only one positive sample was found by this method, corresponding to a prevalence of 0.7% of the studied population. The rate of brucellosis in the studied population is 704 per 100,000 inhabitants, despite the knowledge that about the brucellosis have both slaughterhouse workers and milkers.

1181

AEROBIC BACTERIAL CAUSES OF WOUND INFECTIONS IN PATIENTS AT A CAMBODIAN SURGICAL CENTER FROM 2011-2013

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Microbial infections delay wound healing, but the identification of pathogenic bacteria in infected wounds is often unknown. In this study, anonymous wound specimens were obtained from Children's Surgical Center, a Non-Governmental-Organization Hospital located in Phnom Penh, Cambodia. The main objective of this study was to use standard culture and molecular based identification methodologies to discover the prominent populations of pathogenic bacteria associated with wounds in Cambodia and characterize their antibiotic resistance profiles. This study represents an initial effort to develop longitudinal wound culture information across Cambodia to provide estimates of causative agents, evolving antimicrobial-resistance patterns (with particular emphasis on Multi-Drug Resistant Organisms (MDROs)), and the relative proportion of various genotypes for isolated MDROs. Genotypic analysis of selected isolates of interest will be performed in the near future. Specimens from open and closed wounds from a wide variety of anatomical locations, and rarely from sterile body sites, were received from October 2011 to April 2013. Specimens were sent to NAMRU-2 PP based on the clinical judgment of CSC surgeons and not on any set criteria. NAMRU-2 PP was not given any clinical information except for a brief description of the specimen's anatomical source. A total of 252 specimens were collected with 176 positive and 75 negative results by standard bacterial culture, respectively. *Staphlycoccus aureus* was the most frequently isolated bacteria with significant resistance to Methicillin demonstrated. A host of other clinically important Gram positive and Gram negative bacteria were also isolated. Significant resistance to extended spectrum beta-lactam antibiotics was demonstrated among clinically important Gram negative bacteria. The only observed resistance to carbapenems was observed among two isolated strains of *Acinetobacter spp.* Culturing for anaerobic bacteria was not performed. The results of this study demonstrated a significant presence MDROs at one hospital in Cambodia.

1182

ESTABLISHING A PREVALENCE STUDY OF GI PATHOGENS IN A GUATEMALAN COMMUNITY: RESULTS FROM THE PILOT STUDY

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Gastrointestinal pathogens account for a large burden of disease in developing countries. *Ascaris lumbricoides* infection can lead to malnutrition, cognitive delay and intestinal perforation. *Helicobacter pylori,* meanwhile, can result in chronic gastritis and gastric carcinoma in long-term infection. The interaction between these common pathogens is unclear at present, however it has been postulated that helminthiasis alters the inflammatory response to *H. pylori* and decreases gastric cancer risk. Estimates of the prevalence of *A. lumbricoides* in Guatemala range from 17.1% to 40%. More data is required on the prevalence of *H. pylori* in Guatemala, however one study in children revealed a rate of 20% and Guatemala reports the second highest rate of gastric cancer in the world. This pilot aimed to gather preliminary data on the prevalence of the two pathogens. Patients over one year and attending the Mayan Medical Aid clinic (Santa Cruz, Atitlan) were selected. A survey was completed before routine clinic visits. Fresh stool samples were brought to the clinic and infection was determined using fecal smear and rapid antigen test (for *A. lumbricoides* and *H. pylori* respectively). Participants returned within 7 days to obtain test results and treatment. 73 patients were enrolled in the study and completed the initial survey, with 56 (77%) returning stool samples. Of these, 57% returned the following week for follow-up. 5/50 (10%) samples tested positive for *A. lumbricoides* whilst 14/52 (27%) tested positive for *H. pylori*. The average age of *A. lumbricoides* cases was 13 years (median 2). Conversely, the average age of *H. pylori* cases was 23 years (median 23). No cases of co-infection were detected. These results may reflect higher *A. lumbricoides* prevalence among children and higher *H. pylori* prevalence among adults. It may be necessary to include *A. lumbricoides* immune tests in future studies to determine the interactions between these two pathogens. Subjectively, the study was well accepted in the community and future large-scale prevalence studies would be viable.

1183

INFLUENZA ILLNESS AMONG CASE-PATIENTS WHO MEET THE SEVERE DENGUE CASE-DEFINITION, EL SALVADOR, 2012

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Concurrent infection influenza-dengue was described during the 2009-10 influenza pandemic. Was no systematic detection and, in some cases, unspecific diagnostic tools were used. Our objective was to estimate the prevalence of concurrent infection by influenza and dengue virus and identify influenza cases among suspected cases of dengue in patients hospitalized for severe acute respiratory infection (SARI), or suspicion of dengue in the Salvador. We studied 321 subjects of all ages hospitalized who complied with either the severe acute respiratory infection (SARI) case definition or the severe dengue case definition and sought care at influenza sentinel hospitals in El Salvador during July 5-September 30, 2012. This period is considered higher activity of both viruses in the country. All cases were tested for influenza and dengue viruses at El Salvador's National Influenza Center using real time RT-PCR. Ten percent (10%) of all SARI cases and nineteen percent (19%) of all severe dengue cases were positive for influenza virus and negative for dengue virus. One percent of participants had co-infections with influenza and dengue viruses. Co-infection occurred more frequently among dengue case-patients than among SARI case-patients (1.6% vs. 0.5%). It has been shown that concurrent infection of influenza and dengue occurred in the period of greatest viral circulation of both viruses in El Salvador, and outside the framework of the pandemic. We also found that influenza is an important differential diagnosis to consider in patients hospitalized for suspected dengue. Based on these findings we recommended consider empirically treating SARI case-patients with oseltamivir within 48 hours of symptom onset during influenza season, even if they also meet the severe dengue case-definitions. Also consider taking nasal swabs for all severe dengue cases hospitalized during influenza season. It can increase the number of influenza positive cases admitted in hospital.

1184

A RARE CASE: ACUTE TRANSVERSE MYELITIS FROM GNATHOSOMIASIS

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The cutaneous gnathostomiasis is the commonest parasitosis in Southeast Asia but the neurological gnathostomiasis is quite rare. This is a rare case of spinal cord gnathosomiasis. An 86 years old, Thai man presented with sudden and rapid progression of paraplegia and sensory loss below the nipple line with acute urinary retention for 2 hours before consultation. This patient had never eaten raw meat and fish and also had no history of migratory swelling. The physical examination revealed conscious, coherent, oriented, without cardiopulmonary distress; intact cranial nerves; paraplegia of both lower extremities; decreased sensation below T4 level; with no response of deep tendon reflex and absent of Babinski's reflex. The complete blood count revealed no eosinophilia. Blood biochemistry tests, urinalysis, stool examination, chest X-ray and plain whole spine X-ray did not show any abnormalities. However, the Magnetic Resonance Imaging showed hypersignal T2W without enhancing at thoracic level which was diagnosed either neuromyelitis optica or acute myelitis. The lumbar puncture was performed with the following results: WBC 3 cells/uL, RBC 50 cells/uL, and normal protein and sugar levels. Both cerebrospinal fluid (CSF) and serum for *Gnathostoma* antibody (Western Blot) were positive. Albendazole 400 mg/day for 14 days and pulse methylprednisolone 1 g/day for 5 days was started. He gradually improved sensation and bowel and bladder movement, while motor strength had little improvement to grade II/V.

1185

EVALUATION OF WEST NILE VIRUS AND EPSTEIN-BARR VIRUS AS CAUSES OF ACUTE MENINGITIS AND ENCEPHALITIS IN THE COUNTRY OF GEORGIA

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There is limited information available regarding infectious etiologies of central nervous system (CNS) infections in the country of Georgia. Data on the relative frequency of these pathogens are important for diagnosis, clinical management, and public health decision making. While West Nile virus (WNV) IgG antibodies have been detected in human sera, there have been no serologically or virologically confirmed cases of WNV in Georgia, and while Epstein-Barr virus is a recognized cause of CNS infections in Georgia, the frequency of EBV-related meningitis or encephalitis is unknown. In October 2010, a hospital-based surveillance study was initiated in Tbilisi, Georgia to determine the infectious etiologies of acute meningitis and encephalitis, and to enhance laboratory capacity for the diagnosis of CNS infections. Cerebrospinal fluid (CSF) and serum (acute and convalescent) were collected. ELISA was used to test for WNV IgM in CSF and serum, and EBV IgM in serum only. Of 199 patients enrolled as of April 2013, 111 were children (age six months - 17 years) and 93 were males. The clinical diagnoses for these patients were bacterial meningitis (82), viral meningitis (95), TB meningitis (9), and encephalitis (13). Of the 199 enrolled patients, samples from 129 have been tested. Of these 129 patients, two were borderline positive (titers of 1.06 and 0.079) for WNV, and 17 patients' serum samples were positive for EBV (titers ranging from 1.2 to 8.1). Of the 17 EBV positive patients 12 were clinically diagnosed as bacterial meningitis, two as viral meningitis, and three as viral encephalitis. Of the 17 EBV positive patients nine had a negative acute test. Continued surveillance is important in order to further define the causes of acute CNS infections in Georgia, however the initial findings of this study provide valuable baseline information regarding the current and emerging etiologies of CNS infection in Georgia.

1186

BURDEN, EPIDEMIOLOGY AND SEASONALITY OF CHOLERA AND ROTAVIRUS AMONG PATIENTS WITH ACUTE DIARRHEA IN FOUR HOSPITALS IN HAITI, 2012-2013

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An outbreak of cholera began in Haiti in October 2010, and by March 2013 had caused over 650,000 reported cases. All patients with acute watery diarrhea are considered cholera patients for surveillance purposes, but laboratory testing is not performed systematically. Although little is known about the burden of rotavirus in Haiti, national introduction of rotavirus vaccine is planned for 2013. In April 2012, we initiated laboratory-enhanced surveillance for diarrheal disease in patients admitted to 4 Haitian hospitals in 3 departments: West, Artibonite, and Southeast. Nurses collected stool specimens and administered a questionnaire to patients with ≥3 episodes of acute watery diarrhea per day and onset in the past 7 days, who had not received antibiotics. Stool was sent to the National Public Health Laboratory, cultured for *Vibrio species*, *Salmonella* and *Shigella*, and tested for rotavirus by ELISA. From April 3, 2012 – February 27, 2013, we collected specimens from 1,484 patients. The mean age was 27 (range: <1 month – 95 years), 47% were female and 86% were treated in Cholera Treatment Facilities (CTFs). Overall, 973 (65.6%) specimens yielded *V. cholerae*, 8 (0.5%) *Shigella*, and 6 (0.4%) *Salmonella*; 43 (2.9%) were positive for rotavirus. Among 281 children <5 years old, 28.5% and 10.7% were positive for *V. cholerae* and rotavirus, respectively, compared to 74.2% and 1.0% in 1,186 patients ≥ 5 years old. Cholera was more common than rotavirus among 120 children admitted to CTFs (57.5% vs. 6.7%), but not among 150 children admitted to pediatric wards (6.0% vs. 13.3%). Of 973 *V. cholerae* isolates, 970 (99.7%) were serotype Ogawa and 3 (0.3%) were serotype Inaba. The proportion of specimens yielding *V. cholerae* ranged from 43% – 80% per month and was highest during May—June and November—December, 2012. The proportion positive for rotavirus per month ranged from 0 – 17% and was highest in February, 2013. In Haiti, cholera continues to be a major cause of hospitalizations for diarrhea in children and adults, and rotavirus is a common cause of diarrhea among children <5.

1187

VEGFR3 AND OTHER ANGIOGENIC/LYMPHANGIOGENIC GENES ARE ASSOCIATED WITH DEVELOPMENT OF LYMPHEDEMA OF FILARIAL ORIGIN

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Pathologies of lymphatic filariasis such as lymphedema (LE) and hydrocele are observed in a subset of individuals in endemic areas even though all inhabitants in an endemic community have equal chance of being inoculated with the parasite. While there is ample evidence suggesting that pathology of filarial diseases have genetic propensity, very little work has been done to address it. Recent studies on the molecular mechanisms controlling the lymphatic vessels have shown that vascular endothelial growth factors control lymphangiogenesis in humans by activating the VEGF receptor-3 (VEGFR3) which is principally restricted to the lymphatic endothelium in adults. We showed recently that soluble VEGFR3 in the blood correlated with development of LE and reducing it with doxycycline treatment has ameliorating effect on LE. However, its genetic basis was not known. To assess the role of VEGFR3 and other lymphangiogenic/angiogenic polymorphisms in LE development, a cross-sectional study was designed to genotype 1303 unrelated Ghanaian volunteers comprising 266 lymphedema patients, 691 infected patients but without pathology and 346 endemic controls for single nucleotide polymorphisms (SNPs). In all, 132 SNPs from 64 genes were genotyped and analyzed. A single marker analysis done showed associations with 7 SNPs which included: VEGFR3 [P=0.04], insulin like growth factor-1 (IGF-1) [P=0.03], matrix metallopeptidase 2 (MMP-2) [P=0.03], nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (NFKB- inhibitior alpha) [P=0.001], carcinoembryonic antigen-related cell adhesion molecule 1(CEACAM-1) [P=0.03], tissue inhibitor of metallopeptidase 2 (TIMP) [P=0.004] genes and interleukin 17 [P=0.05]. Apart from the interleukin 17, all the other mentioned genes were associated with lymphangiogenic/angiogenic modulations, which is a hint that lymphedema development may be associated with lymphangiogenic/angiogenic factors.

1188

THE IMPACT OF THE RAMADAN PERIOD ON CLINICAL PARAMETERS OF PATIENTS SEEN AT A DIARRHEAL HOSPITAL IN URBAN DHAKA, BANGLADESH, 1996-2012

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Ramadan is a month in the Islamic calendar when a significant portion of the global community does not drink or eat from before sunrise to sunset. We hypothesized that such changes may either impact the identity of infecting enteric pathogens, or affect health seeking behavior once ill. To investigate this, we used prospectively-collected data from the Diarrheal Disease Surveillance System of the Dhaka Hospital of the icddr,b in Bangladesh, where 90% of the population are Muslims. For the years 1996-2012, we compared the etiology and clinical presentation of patients who presented with diarrhea during the Ramadan period with patients who presented during control periods, defined as the 30-day period immediately prior to Ramadan. Infecting pathogens were largely the same, although conventional stool cultures obtained from non-Ramadan patients were more likely to yield *Shigella spp.* than during Ramadan periods. Among adult Muslims, Ramadan patients were more likely to complain of severe thirst on arrival compared to controls, though there were no significant differences in rates of severe dehydration between groups. Since cholera is endemic in this area, and can have acute onset that can rapidly lead to severe dehydration, we performed a cholera-based sub-analysis, comparing adult Muslim patients with confirmed *Vibrio cholerae* O1 or O139 infection who presented during Ramadan with those who presented during control periods. Cholera patients who presented during Ramadan were older and more likely to complain of severe thirst. Those who arrived after sunset during Ramadan had a shorter duration of diarrhea, and higher rates of severe thirst, drowsiness, and severe dehydration compared to those arriving after sunset during control periods. These differences were not seen when comparing daytime Ramadan and daytime control arrivals. Our findings suggest that Ramadan may affect both the profile of enteric pathogens, and clinical features of those seeking medical care for diarrhea during Ramadan.

1189

CLUSTER INVESTIGATION OF MELIOIDOSIS CASES REVEALS EVIDENCE OF ENDEMICITY IN PUERTO RICO

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Six locally-acquired melioidosis cases have been identified in Puerto Rico since 1982; of these, four (67%) were fatal. We conducted cluster investigations of the most recent cases (2010 and 2012) to determine likely locations of exposure and identify risk factors for seropositivity among case-patient contacts. Neighbors, co-workers, and social contacts of case-patients were interviewed to collect demographic data, behavioral practices, and past and current medical history. Serum samples were tested for evidence of prior exposure to *Burkholderia pseudomallei* by indirect hemagglutination (IHA); seropositive was defined as IHA titer ≥1:40. For the 2010 case, three (7%) of 43 neighborhood contacts and zero (0%) of eight coworkers were seropositive. For the 2012 case, who was an intravenous drug user (IVDU), 13 (23%) of 56 neighborhood contacts, zero (0%) of one co-worker, and two (67%) of three IVDU contacts were seropositive. Risk factors significantly associated with seropositivity were having had wounds, sores or ulcers (Odds Ratio [OR] =4.7; 95% confidence interval [CI]: 1.3-17.0) and current or past use of illicit drugs (OR=4.2; 95% CI: 1.3-14.0), specifically cocaine (OR=9.1; 95% CI: 1.4-59.0). Traditional risk factors for developing disease such as age, travel history, smoking, alcohol consumption, co-morbidities, and soil and water exposures were not associated with seropositivity. Although only sporadically reported since 1982, the high rate of seropositivity identified in this investigation suggests regional endemicity of *B. pseudomallei* in Puerto Rico. Additional studies are planned to conduct spatial analysis of geospatial data and to identify additional cases and infection distribution through serologic examination and hospital chart reviews. Awareness campaigns addressing melioidosis should be developed for clinicians, laboratories, and high risk populations to promote early recognition and appropriate treatment to help reduce morbidity and mortality.

1190

MANAGING ACUTE FEBRILE ILLNESS IN THE COMMUNITY - IMPLICATIONS FOR POLICY AND PRACTICE IN THE ERA OF RAPID DIAGNOSTICS TESTS FOR MALARIA

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Febrile illness like malaria and pneumonia are among the leading causes of child death in Sub-Saharan Africa (SSA). Fever in the tropics has often been considered to be primarily due to malaria and has been treated as such. Recently the treatment guidelines have been further elaborated, recommending parasitological diagnosis before treatment with antimalarials. Pneumonia is still diagnosed presumptively based on symptoms of cough or difficult breathing with fast breathing. At community level, malaria rapid diagnostic tests (mRDTs) were included in Integrated Community Case Management of sick children (ICCM) algorithms where community health workers (CHWs) classify symptoms of malaria, pneumonia, diarrhoea, and treat with antimalarials, antibiotics, oral rehydration solution (ORS) and zinc. The overall aim of this study was to document the diagnoses and treatments prescribed to children under five by CHWs under routine ICCM in Uganda. Patient registries for Jan-Dec 2012 were collected from 106 CHWs in 5 districts in Mid West Uganda. The proportion of child consultations where the child was diagnosed with malaria, pneumonia or diarrhoea was calculated. Quality of care was assessed per each sick-child episode based on treatment provided. A total of 4515 child consultations were documented. Out of these 70% presented with fever and 52% were malaria positive (78% of all fever cases). 48% of children had symptoms of pneumonia, of which 35% were also positive for malaria. Approximately half of the children with pneumonia had fever. 13% were diagnosed with diarrhoea, whereof 29% also were positive for malaria and 34% had pneumonia. Only 2% had overlapping symptoms of diarrhoea, malaria and pneumonia. Of the children with confirmed malaria, 67% were treated with artemisinin combination therapy (ACT); 92% of children with pneumonia received amoxicillin; and 83% of diarrhoeas received ORS and zinc. Only 10% of children with a negative malaria test were prescribed ACT and 6% of children without fast breathing were prescribed antibiotics. With the rapid expansion of ICCM across countries in Africa, the consideration whether to include mRDTs in the clinical algorithm is key. Our findings show that symptom overlap is common and the majority of children receive more than one diagnosis. mRDTs reduced unnecessary malaria treatment and likely increased appropriate treatment of children with non-malaria fever. Correct treatment by CHWs was high.

1191

TOSCANA VIRUS INFECTIONS IN MARSEILLE, FRANCE: A STUDY OF SEVENTEEN CLINICAL CASES WITH LABORATORY DOCUMENTATION

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Toscana virus (TOSV) is a sandfly-borne phlebovirus (Phlebovirus, Bunyaviridae), discovered in 1971 in Central Italy, principally transmitted by *Phlebotomus* perniciosus and Ph. perfiliewi. TOSV is considered as an emerging virus in Portugal, Spain, France, Italy, Greece, Cyprus, Croatia, and Turkey. Most of the clinical reports of TOSV infections are case reports, and few studies considered series of cases. Moreover, the laboratory documentation of suspect cases is rarely analysed by using the WHO criteria for arboviral infections (2012 Case Definitions: Nationally Notifiable Conditions Infectious and Non-Infectious Case. (2012). Atlanta, GA: Centers for Disease Control and Prevention). TOSV infection was diagnosed based on virus isolation (n=1), RT-PCR (n=6), TOSV IgM (n=15) and or seroconversion (n=6). Here, we report a series of 17 clinical cases (15 confirmed, 2 probable) of TOSV infection recorded in the Virology laboratory of the Public Hospitals of Marseille between 2004 and 2011. Eleven patients were men (64.7%), and median age was 45 years (range, 4-76). Fourteen TOSV infections (82%) occurred between June and September, and 3 cases in March, April and November; only two patients reported recent travel in Croatia and in Tuscany, respectively. The outcome was favorable in all cases. All cases were symptomatic, and CNS signs were observed frequently: meningitis, meningoencephalitis or encephalitis (n = 10), atypical neuro-muscular symptoms (n = 3) such as hemiparesis, fasciitis, myositis. In conclusion, TOSV infection is a prominent cause of summer febrile illness with or without central or peripheral neurological manifestations in south-eastern France. In our study TOSV was the third cause of CNS infection, after enteroviruses and herpesviruses (HSV and VZV). These findings, together with seroprevalence data, confirm that TOSV is the most frequent arbovirus in France.

1192

CASE REPORT: INFECTION WITH *LEISHMANIA (VIANNIA) LAINSONI* IN LORETO, PERU

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Leishmania (Viannia) lainsoni has been isolated in human samples, sandflies and putative reservoirs in the Amazon Basin of Brazil, in the Yungas of Bolivia, and in the central and southern Peruvian Amazon Basin. However, this species has neither been reported in the northern Peruvian Amazon basin nor other countries further north such as Ecuador. We describe here a recent case of *L. (V.) lainsoni* from a 47 year old male patient in Loreto, Perú. The patient had a 13 x 8 mm ulcer on the forehead close to the right ear presenting with neck lymphadenitis. There was neither mucosal compromise nor previous report of leishmaniasis. The patient has lived in Loreto since 2009, but travels continually for work. Before diagnosis, he worked in Quillabamba, Cuzco (Jul 2012) but only within city limits where there have been no documented reports of leishmaniasis. Then he worked in Coca, Ecuador (Aug-Oct 2012) and finally returned to work in rural areas of Loreto (Oct 2012-Feb 2013) when the lesion appeared. Both microscopy and culture of lesion aspirates were positive for *Leishmania*. PCR for kinetoplastid DNA from a punch biopsy, scraping and filter paper impression confirmed the diagnosis. In addition, our validated nested RT-PCR identified the species as *L. (V.) lainsoni*. The patient received complete treatment with 20 daily doses of intravenous sodium stibogluconate (20mg/kg) over 26 days and achieved 70% lesion healing by the end of treatment. This patient was most likely infected in Loreto, Peru, or Coca, Ecuador, although it cannot be conclusively demonstrated that he was not infected in the Southern Amazon Basin of Peru. Our finding suggests that the known geographical distribution of this uncommon *Leishmania* species is broader than currently suspected, and highlights the need to continue identifying the species of *Leishmania* circulating in the region.

1193

EARLY PARASITOLOGICAL RESPONSE IN PATIENTS TREATED WITH ARTEMISININ COMBINATION THERAPIES IN ASIA: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Delayed early parasite clearance is the hallmark of artemisinin resistance. The proportion of patients remaining parasitaemic on day 3 is a useful indicator of early parasitological response. The WHO currently uses positivity rates on day 3 exceeding 10% as an alert for a potential decline in artemisinin sensitivity. We investigated the clinical determinants associated with persistent parasitaemia on day 3 and explored the trends of early parasitological response following the initiation of artemisinin-based combination therapies (ACT) treatment in Asian patients. A total of 10,828 individual patient data from 26 efficacy trials (1991-2010) shared with the Worldwide Antimalarial Resistance Network (WWARN) were pooled using standardised methodology. Overall 5362 (49.5%) patients were treated with Artesunate Mefloquine (AS+MQ), 2,726 (25.2%) with Dihydroartemisinin-Piperaquine (DP), 2,415 (22.3%) with Artemether-Lumefantrine (AL), and 325 (3%) with Artesunate-Amodiaquine (AS-AQ). Two major risk factors were identified for being parasitaemic on Day 1, 2 and 3: the log of the baseline parasitaemia and being febrile at enrolment (both $p<0.001$). In multivariable analysis in which study site was fitted as a random effect, the risk of being parasitaemic on day 1 [AOR: 0.93, 95% CI: 0.85-1.01, P=0.10], day 2 [AOR: 0.86, 95% CI: 0.78-0.96, P=0.01] and day 3 [AOR: 0.94, 95% CI: 0.84-1.04, P=0.23] were found to have decreased over time after adjusting for age, baseline parasitaemia (log), initial fever and the treatment received. The risk of being parasitaemic on day 3 did not differ between treatment regimens. However this pooled analysis didn't include data from Thai-Cambodia border where artemisinin resistance has emerged.

1194

INFECTIOUS ETIOLOGIES OF MALARIA-NEGATIVE ACUTE FEBRILE ILLNESS AMONG ADULTS AND CHILDREN IN NORTHEAST TANZANIA

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The most recent WHO Guidelines recommend the use of parasitological testing to guide antimalarial drug use in all ages. To support this and guide management of test-negative patients there is an urgent need to know more about aetiologies to non-malarial febrile illness. We aimed to identify the cause of non-malarial fevers in children and adults in a malaria-endemic area of northeast Tanzania. 1087 patients, 3 months to 50 years old, with a history of fever during the past 48 hours were enrolled. A combined throat and per nasal swab, urine sample and aerobic blood culture were taken on each case. Chest X-ray was done in cases with suspected pneumonia. Patients were followed up 2, 7 and 14 days after enrolment to follow progress and to take a convalescent blood sample. Blood and NP-swabs were analysed by culture growth and multiplex PCR to identify illness-causing pathogen(s). Upper respiratory tract infection was the most common diagnosis in children under five years (67%) while adults were most commonly diagnosed with unspecified fever (34%) and urinary tract infection (33%). 8% of children below five were diagnosed with non-severe pneumonia and 4% with severe pneumonia. 46 patients were admitted (4%) and all but five of them were less than five years. There were no case fatalities during the study and 1010 patients were seen on day14 whereof 2% reported no improvement or worsening. 19% of throat/NP cultures were positive with most (63%) growing *S. pneumonia*. Positive growth was more common among children under five (33% positive cultures) compared to older patients (OR 1.63). For urine cultures, 9% of children less than one presented significant bacterial growth, all with *E.coli*. In adults, 6% had significant bacteria in urine, most of them *E.coli* or *klebsiella*. Seven blood cultures flagged positive for *Salmonella typhi*, four children grew *streptococci spp* and two adult blood cultures were positive for *E.coli*. Results from multiplex PCR and X-rays will provide further understanding of the illness causing pathogens and inform management guidelines.

1195

A TROPICAL DISEASE IN CLEVELAND, OHIO: A CASE OF CEREBRAL MALARIA

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Severe *Plasmodium falciparum* malaria has a high mortality rate often presenting in the first 48 hours after hospital admission, thus correct diagnosis and delivery of appropriate treatment can be life- saving. Artesunate is the recommended treatment but parenteral therapy is only available through the Centers for Disease Control. Providers who do not see this disease severity can often misdiagnose or delay diagnosis.

We present a case of a 67 y/o Caucasian male admitted to the Veteran's Hospital in Cleveland, OH with a past medical history notable for atrial fibrillation presenting with flu-like symptoms, notably diarrhea for the past 3 days after returning from a trip to Uganda where he traveled 6 times previously for mission work. He complained of myalgias, non-bloody emesis, dizziness and chills. He drank only bottled water, had no sick or animal contacts, used mosquito nets and became ill on the plane. The patient did not take malaria prophylaxis nor did he receive any vaccinations prior to travel. On exam he was febrile, tachycardic and tachypneic with no significant physical exam findings. Lab work showed a bilirubin: 2.7, LDH: 562, hemoglobin: 15.9->11.3 and hematocrit: 46.2->32.5, platelets: 87->42, SGOT: 65->93, SGPT: 36 with creatine: 1.2. His blood cultures, HIV, hepatitis and stool cultures were negative. Malaria smear was ordered and atovaquone/proguanil was started. With one dose left before finishing treatment, he had worsening confusion with altered mentation. Labs showed thrombocytopenia and transaminitis. On initial smear he had a 5% *P. falciparum parasitemia* which dropped to 2%. Parenteral Artesunate was requested from the CDC malaria branch. Within in 24hours of starting parenteral Artesunate the patient clinically improved. Whole blood collected and stored in the refrigerator for over 3 days showed a high grade *parasitemia* greater than the patient's smears and the parasite itself, was able to be grown in culture. He had a sustained anemia and there was concern for a post-treatment hemolytic process. We advocate for increased accessibility to parenteral Artesunate therapy, rapid diagnostics and improved malaria educational tools/scoring for clinical assessment and progression to advanced disease for clinicians/house officers in the United States unfamiliar with this disease and severe manifestions such as cerebral malaria as well as the presenting symptoms and clinical course.

1196

INNOVATIVE AND FAST ASSAYS FOR THE DIAGNOSIS OF *SCHISTOSOMA MANSONI* FOR CLINICAL ACUTE AND/OR CHRONIC FORMS

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Control constraints of schistosomiasis include the lack of diagnostic methods with high sensitivity. We initiated a prospective study in southeast Brazil in order to develop sensitive diagnostic methods for *Schistosoma* mansoni infection, with 4 endemic areas together with 80 travelers infected in a freshwater pool. Sera, whole blood, urine and saliva samples from the patients were used for the standardization of innovative diagnostic methods. Comparisons were performed with eggs in feces, IgG titers, encephalomyelitis by NMR and clinical symptoms. The new methods used were immunochromatography (dipstick), Immunomagnetic Separation and ELISA with highly purified monoclonal antibodies. We could diagnose acute patients 10 days post-infection, also more than 95% of positive cases from chronic and low endemicity patients. New methods for IgG detection using purified glycoprotein or recombinant protein or peptides (10 aminoacids) were superior to conventional ELISA. Best results were seen for recombinant protein with 100% of sensitivity. Data showed 100% of sensitivity of chronic patients and 98% of acute patients.

1197

CLINICAL FEATURES OF NON-COMPLICATED MALARIA IN SEMI-IMMUNE INDIVIDUALS OF WESTERN BRAZILIAN AMAZON

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We analyzed clinical characteristics of 115 microscopy and PCR-confirmed, uncomplicated, symptomatic malaria cases diagnosed in urban Amazonians through passive detection in Acre, Brazil. Patients were between 6 years and 92 years (58.2% males), and were highly exposed to malaria (68.1% had between 1 and 10 previous episodes, 29.2% had more than 10 previous episodes and only 2.7% never had an episode before). The most common species was *P. vivax* (73.04%), followed by *falciparum* malaria (25.2%) and mixed-species infection (1.76%). Parasitemia was below 500 parasites/mm^3 in 64% of cases, and only 1% of cases had *parasitemia* higher than 10.000 parasites/mm^3. Eighteen symptoms (fever, chills, sweating, headache, retro-ocular pain, myalgia, arthralgia, abdominal pain, lumbar pain, nausea, vomiting, diarrhoea, dizziness, flu-like symptoms, cough, dyspnea, odynophagia, bitter mouth) were investigated using a structured questionnaire. Headache (89.4%), fever (84.1%) and chills (77.0%) were the most frequent symptoms. Fever was perceived as "intense" in 47.0% episodes, with no fever reported in 15.9% episodes, although other symptoms were present. Falciparum malaria cases were more likely to present with gastrointestinal symptoms, although no statistical significance was obtained. Arthralgia and myalgia were more frequent in older people (100% of upper age quartile against 70% of lower age quartile, $p < 0.01$, Qui-square test) than younger people, while respiratory symptoms were more frequent in the younger (66.7% in the lower age quartile, $p = 0.025$, Qui-square test). Diarrhoea was also more frequent in the upper age (10.7%) than lower age (3.3%) quartile ($p = 0.01$, Qui-square test). The only symptom that correlated with higher peripheral *parasitemia* was dizziness (70.7% versus 48.6%, $p = 0.03$, Qui-square test). No significant association was found between symptoms and sex or previous malaria exposure. These factors are all likely to affect the effectiveness of malaria control strategies based on active or passive detection of febrile cases in semi-immune populations.

1198

BIOMARKERS AND RISK FACTORS FOR INADEQUATE ERYTHROPOIESIS IN ALL-CAUSE PEDIATRIC ANEMIA IN MOZAMBIQUE

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Anemia affects 1.6 million people globally and is massively understudied, particularly as a multifactorial disease. Dysregulation of erythropoiesis is thought to be a major factor in chronic and recurring anemias, but is rarely studied in human populations, because of poor accessibility of bone marrow and limitations of methods for measuring erythropoiesis. We collected 286 bone marrow (BM) aspirates in the context of a large case-control study of anemia in Mozambique and examined a wide range of clinical, hematological, biochemical, immunological, microbiological

and genetic markers. We developed novel flow-cytometric analysis and a transcriptional profile based on erythroblast-specific gene expression to quantify erythropoiesis. Both measures correlated well with classic morphological quantification in stained BM smears (both $p<0.0001$). Whereas peripheral reticulocyte numbers showed no correlation with BM erythropoiesis in this cohort, the red cell distribution width (RDW), a measure of variance in erythrocyte volume, was validated as a novel peripheral biomarker for this important response ($p<0.0001$). Samples were categorized as having low or high erythropoiesis relative to hemoglobin levels. Uni- and multivariate statistical testing revealed that high C-reactive protein levels and *bacteremia* were most highly associated with low erythropoiesis. Hemolysis, shifts in the BM balance of myeloid cells and lymphocytes and high EPO levels relative to the degree of anemia were associated with high erythropoiesis and unexpectedly, so was infection with *Plasmodium falciparum*. The risk factor panel for low erythropoiesis differed from the panel of risk factors for anemia identified in the same cohort, in particular in regards to nutritional deficiencies. Thus, these data provide superior direct and peripheral biomarkers of erythropoiesis, differentiate several groups of erythopoietically suppressed BM and provide a rich data set for our ongoing analysis of transcriptional footprints indicative of underlying mechanisms. Such findings may inform future therapies for this serious disease.

1199

HERPES ZOSTER ASSOCIATED WITH PENTAVALENT ANTIMONY THERAPY FOR TEGUMENTARY LEISHMANIASIS - A 22 CASE SERIES OF A TROPICAL MEDICINE HEALTH SERVICE IN BRAZIL

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The pentavalent antimonial is considered as the first line drug for leishmaniasis treatment in Brazil. Although it has been prescribed for many years, the mechanism of action is not completely understood. It is known antimonial inhibit intracellular glycolysis and fatty acid oxidation, beside link with sulfhydryl groups, resulting in the parasite death. Side effects are frequent and include myalgia, arthralgia, anorexia, headache, vomiting, and even cardiotoxicity, nephrotoxicity and sudden death are possible. Herpes Zoster (HZ) has been rarely described during or shortly after receiving therapy with antimonial. HZ is a secondary clinical manifestation of varicella zoster virus (VZV), an α-herpes virus that seems associated with depressed cellular immunity, and eventually related with antimonial treatment. This paper reports a series of cases of HZ observed during or shortly after antimonial treatment for the various clinical forms of leishmaniasis. Patients reviewed were treated at the infectious disease clinic of a Brazillian University Hospital, between 1995 and 2010. The onset day of HZ was recorded. Out of 21 in a total of 22 patients were male with a median age of 53. Sixteen had Tegumentary Leishmaniasis (LC, LM, Disseminated). N-methyl-glucamine was used in 18 cases and Sodium Stiboglucanate in 4. The median dose of N-methylglucamine was 18.2 mg / kg. The median time to onset HZ was 20 days (25 days for N-methyl and 16.5 to stibogluconate). Although the mechanism by which varicella zoster virus becomes reactivated is not known, the association of zoster with conditions that depress cellular immunity led to suppose that patients under antimonial treatment could be transiently suppressed regarding cellular immunity even considering that older age and high doses of antimony can act as predisposing factors. Most of our patients had tegumentary leishmaniasis and their base clinical conditions cannot be related an immunological suppression.

1200

THE CHANGING FACE OF NOMA IN LAOS

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Noma is an orofacial gangrene and an opportunistic infection, primarily occurring in children whose health is weakened by malnutrition and living in extreme poverty. Acute noma rapidly destroys the face and, without treatment, has a high mortality of 90%. In the acute phase the patient is treated with antibiotics and nutritional support. Survivors of noma are often severely disfigured and functionally impaired (trismus, speech problems, oral incontinence). Surgical rehabilitation of noma patients requires experienced surgical teams. Noma cases have been recorded historically throughout the world, but economic and public health improvements have resulted in the apparent disappearance of noma in developed countries. Most cases of noma occur on in Africa. There are few reports of noma in Asia. Prior to 2002, there were no reported cases of noma in Laos. Since 2002 one patient with acute noma and 36 survivors have been found throughout the country. Visiting surgical teams have successfully treated the majority of these patients. Most are young adults whose disease occurred several decades ago, which suggests that noma is disappearing in Laos. Despite economic growth, chronic malnutrition continues to weaken the health of almost 50% of Lao children with much higher rates in rural areas. These rates have changed little despite economic improvements. The risk factors for noma are malnutrition, poverty, poor hygiene and lack of clean water, lack of breastfeeding, infections especially measles and malaria, close contact with animals, lack of immunizations, vitamin deficiencies and poor health care access. These risk factors continue to threaten the health and well-being of Lao children. Follow-up of Lao noma patients, some for 10 years, reveal that many have required multiple surgeries. This has resulted in improved cosmetic, functional, and psychosocial outcomes. Ultimately, the elimination of noma risk factors should lead to the complete disappearance of this neglected childhood disease.

1201

HISTORICAL OVERVIEW OF STUDIES ON JAPANESE ENCEPHALITIS VIRUS IN NORTHERN VIETNAM, 1964-1978

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In northern Vietnam from April to September every year, children often have had a malignant syndrome called Acute Encephalitis Syndrome (AES). From 1965 to 1977, the total number of AES in children was 22,545 cases, from whom 4,774 died. Before 1964, the etiology of AES in northern Vietnam has not yet been identified; so the JE infection in our country is still a blank area on the World Map. In this study, we have to determine the etiology of the AES by carrying-out: Virus isolation: From 1964 to 1976, we have isolated 22 JEV from different specimens including: 10 from AES children (1 from blood, 9 from brains), 1 from bird, 1 from swine blood, 10 from mosquitoes. The positive diagnoses (HI test) using paired sera taken from AES children have been varied from 25.58% to 67.5%. Investigate the JEV circulation through serological survey of healthy humans: During 1964-1978, we have tested 7,868 sera, from which the positive rate varied from 25% to 82.94%. Vectors transmitting JEV: JEVs were isolated for the first time in 1971 from *Culex tritaeniorhynchus*. Then in 1975-1976, JEV were discovered from *Cx. gelidus, Aedes albopictus* and *Ae. (Sp) diemmaccus*. To this time, *Cx. tritaeniorhynchus* has been identified as the main vector transmitting JEV in northern Vietnam. Explore the circulation of JEV in animals: - Birds: in 1964 we have isolated the strain LD-68 from bird *Garrulax perspicillatus* - Gmelin and have found 8/14 bird species carrying JE antibody; therefore wild birds have played

the role as reservoir of JEV. - Animals: a/ Serological survey in swine: In 1970: 606 pig sera have been collected in several localities, from which 63.69% were positive to JEV with MAT: 1/619.76. b/ Sero-survey in livestock: During 1972-1975, we have continued to investigate sera from other animal species. The results obtained demonstrated that swine was the most commonly infected with JEV to compare with other domestic animals. With the results obtained, we asserted that northern Vietnam was a natural focus of JEV. This virus virtually persisted everywhere, in wild birds, an arthropod transmitted this virus from bird to livestock; at first to a few pigs, *Cx. tritaeniorhynchus* sucking blood from pigs in phase of viremia then widespreading this virus to other pigs, allowed herd of infected pigs increased rapidly and just to this phase, JE infections occur in humans with a small number of children suffered from AES .

1202

STRUCTURAL COMPARISON OF THE ANTIGENIC CHARACTERISTICS OF USUTU VIRUS AND WEST NILE VIRUS ENVELOPE PROTEINS

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Usutu virus (USUV), a flavivirus belonging to the Japanese encephalitis serocomplex, was isolated for the first time from mosquitoes in South Africa in 1959. The virus emerged in Europe in 2001 and caused severe morbidity among two patients in Italy in 2009. A recent study describing the genetic diversity among USUV isolates from Africa showed the existence of an outlier subtype strain named CAR_1969. Cross-reactions observed in serological assays between USUV, CAR_1969 and West Nile virus (WNV) indicate that these viruses share antigenic characteristics amongst their envelope (E) proteins. Therefore, the comparison of their E proteins might provide important information about USUV pathogenesis and immune reactions to the virus in the human body. We investigated the molecular background of the observed cross-reactions by comparing the E protein sequences of seven USUV strains, USUV subtype strain CAR_1969 and WNV strain 2471 with focus on the binding site defined by the WNV neutralizing antibody E16. USUV SouthAfrica_1959 differs from WNV 2741 in three of four residues critical for E16 antibody binding and five of 12 additionally involved residues. In contrast, USUV subtype CAR_1969 differs from WNV 2741 in two critical residues and five additional residues. Furthermore, USUV subtype CAR_1969 differs from other USUV strains in two critical residues. As E16 antibody binding has previously been shown to be highly specific for WNV, the variation in amino acid residues suggests that the region corresponding to the WNV E16 epitope is probably not involved in the observed cross-reactions and might however partially explain the different antigenic characteristics. Nevertheless, as a therapeutic effect of E16 antibody has been described in WNV infected mice, a USUV specific antibody generated against the region corresponding to the WNV E16 binding site might therefore represent an interesting approach for the treatment of USUV infections.

1203

JAPANESE ENCEPHALITIS OUTBREAK IN WESTERN BANGLADESH, 2011

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Acute meningo-encephalitis (ME) surveillance was initiated in October 2007 in four Bangladeshi hospitals, including Rajshahi Medical College Hospital (RMCH) in western Bangladesh. RMCH identified 550 ME cases from October 2007 to December 2008; 33 (6%) were caused by Japanese encephalitis (JE) virus infection. Through the Bangladesh government's event-based surveillance, all Bangladeshi hospitals are required to report unusual increases in ME admissions. In mid-October to mid-November 2011, Kushtia General Hospital (KGH) in western Bangladesh reported an increase in ME admissions over the 0-2 ME cases they usually admit each month. The objective of this investigation was to confirm an ME outbreak and identify the etiology. Both surveillance systems enlisted febrile cases with evidence of acute neurologic dysfunction, including altered mental status. RMCH identified cases throughout 2011, while KGH identified cases from mid-October to mid-November 2011. Physicians collected blood and cerebrospinal fluid (CSF) samples which were tested for JE virus (JEV)-specific IgM. We defined confirmed JE cases as those with detectable IgM against JEV in serum and/or CSF by ELISA. In 2011, RMCH identified 320 ME cases and collected serum and/or CSF samples from 281 (88%). Of these, 39 (14%) had evidence of JEV infection. Median age of JE cases at RMCH was 40 years (range: 4 months - 72 years); 28 (72%) were above 16 years old; 29 (74%) were male. Six (15%) died. 24(62%) JE cases had onset of illness during October to November 2011. The proportion of serum and/or CSF tested ME cases with JE at RMCH was significantly higher in 2011 than 2008 [3% (12/434)], 2009 [2% (11/489)], and 2010 [4% (10/267)] ($p<0.001$ for each year). From mid-October to mid-November 2011, 14 ME cases were identified at KGH. Four cases had detectable IgM against JEV and seven cases did not; although three of them were negative 7-9 days after onset of illness; three case-patients died before blood samples were collected. Median age of confirmed JE cases admitted to KGH was 50 (range: 40 - 60) years; two were male. In conclusion, the high proportion of JE cases among tested ME cases in 2011 in western Bangladesh suggests a JE outbreak. The high proportion of JE cases among adults with ME suggests JE is an emerging infection in this region. The national vaccination programme should evaluate the feasibility of JE vaccination among residents of western Bangladesh.

1204

QUASISPECIES ANALYSIS OF HEPATITIS C VIRUS IN MOTHER-TO-CHILD *IN UTERO* TRANSMISSION

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Mother-to-child-transmission (MTCT) is the most common cause of hepatitis C virus (HCV) infection in children. This study aimed to evaluate viral factors implicated in HCV MTCT. Four HCV-infected pregnant women and one HCV-infected mother-newborn pair were included in this study. Sequences were obtained from the regions 5'UTR, E1, HVR1, E2 and NS5B by direct PCR product sequencing and cloning. Quasispecies diversity was

analyzed by different parameters (clonotype ratio, mutation frequency, Pn and normalized Shannon entropy), comparing (1) MTCT+ vs MTCT- groups, and (2) mother-newborn pair. A framework was used to establish association between nucleotide frequency and MTCT. Two cases of MTCT were identified, but a sample from only one newborn was available. Viral loads from all subjects were above the quantification limit. Both cases of MTCT belonged to genotype 1a and only this subtype was further analyzed. Direct sequencing from PCR products did not reliably represent the quasispecies complexity and was not used. There were no coincident clonotypes between MTCT+ and MTCT- groups, except for 5'UTR. At the amino acid level, mother and newborn shared only the master clonotype. All minor clonotypes were exclusive. Higher quasispecies diversity was observed within E2 and NS5B regions. HVR1 presented the lowest diversity within the coding region. Quasispecies diversity from the MTCT+ group was always greater than seen in the MTCT- group; however, no statistically significance was observed. Thirty-five mutations in the coding regions were significantly associated with MTCT. Data from the mother-newborn pair suggest that the *in utero* transmission occurred in an earlier time point of the pregnancy and that the virus probably crossed the placental tissue leading to a bottleneck. Quasispecies diversity was not associated with MTCT but the presence of significant mutations along the coding region suggests that the whole genome contributes to the ability of *in utero* transmission. Further studies are required to establish if these variants could be useful to predict MTCT.

1205

CLUSTER OF MEASLES CASES AMONG FARMERS CHILDREN, GWABABAWA LOCAL GOVERNMENT AREA, SOKOTO STATE, NIGERIA - 2012

Measles, a highly infectious vaccine preventable disease, associated with high morbidity and mortality. Measles is the fifth leading cause of death among children younger than 5 years in Nigeria. Northern Nigeria suffers recurrent outbreaks due to low immunization intake. On 9 June 2012 we investigated a suspected measles outbreak in Gwadabawa district to identify risk factors and institute control measures. An unmatched Case-Control study was conducted. A case was a person less than five years residing in Gwadabawa presenting with fever, generalized maculopapular rash, plus any of the followings, Fever, Cough, Conjunctivitis, or Coryza between 1 June and 1 July 2012. Neighbourhood controls were used. We administered questionnaires to obtain information on Risk factors, socio-demographic characteristics, and immunization status. Fifteen Blood samples were collected. We interviewed 63 cases and controls 63 controls. The mean age was 25 Months (SD of 14.4) nine samples tested positive to measles IgM. Being vaccinated with one dose of measles and having a national Vaccination card was found to be protective (OR=0.0182, 95% CI: [0.009-0.3532] and OR=0.0067 CI: 0.0059-0.7550] Disease was associated with a history of contact with a known measles case OR=43, 95% CI [12.26-151.87].Children living 5Km within a Health facility were 10 times more like to have Measles (95% CI [1.44-69.26] diarrhea and pneumonia were the commonest complications reported after measles 48% and 16% respectively. Among persons interviewed measles vaccine uptake was 36%. Household income, history of recent travel and overcrowding were not statistically signicantly. In conclusion, measles outbreak was confirmed and risk factors included contact with a measles case, lack of vaccination . Reactive vaccination campaign, prompt case management and Health promotion activities were instituted.

1206

TRENDS IN THE TEMPORAL AND SPATIAL DISTRIBUTION OF YELLOW FEVER IN SOUTH AMERICA

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Yellow fever is a viral infectious disease, endemic in tropical regions of Africa and America. The disease has two transmission patterns, a sylvatic cycle and urban cycle, both leading to clinical manifestations ranging from asymptomatic to mild and severe forms. The sylvatic cycle involves mosquitoes and monkeys. Genetic studies of YFV strains have revealed that strains isolated in South America and Africa are genetically distinct. Nucleotide sequences of envelope region of the genomes of 10 yellow fever virus samples isolated from monkeys, humans or mosquitoes in Brazil between 2008-2010 were determined with the objective of establishing the genotypes and studying the plylogeographical process throughout South America. These fragments were aligned with 60 representative sequences of Yellow Fever, retrieved from GenBank. The Bayesian inference method available in the software BEAST v. 1.6.2 was used in order to analyze the phylogenetic relationship of the strains of this study. Each sequence of the corresponding data set was dated and maximum clade credibility (MCC) tree was generated. The internal nodes were inferred using a Markov Chain Monte Carlo (MCMC) Bayesian approach under a HKY model with a proportion of invariable sites (I), using a relaxed (uncorrelated lognormal) molecular clock. We performed a discrete phylogeographic analysis across 23 localities generating a graphic view of the virus distribution and circulation using SPREAD to plot the geographic coordinates (Latitude and longitude) and GOOGLE EARTH 5.2 to visualization. The obtained MCC Tree suggests a "Source-Sink" pattern of transmission with secondary phylogeographic structure of wave-like transmission as ancient lineages of Yellow Fever are constantly replaced by new ones, and each new introduction causes the seasonal epidemics and epizooties. The discrete phylogeographic analysis suggests that the Amazonian region presents better conditions to maintain viral circulation and diversity and may represent Yellow Fever genetic diversity source.

1207

DEFINING PRODUCT REQUIREMENTS FOR FIELD-DEPLOYABLE YELLOW FEVER TESTS

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The fight against yellow fever could benefit from the development of field-deployable tests. Such tests would have immediate utility for diagnosing individual cases and outbreaks as well as contributing to surveillance and control programs. Tests that are currently available for yellow fever are laboratory based, requiring both good infrastructure and trained personnel, and often have a long turnaround time to results. Field tests performed by minimally trained health staff could provide rapid results, allowing an appropriate response to outbreaks and streamlining of laboratory testing for case confirmation. The authors report results of international and country-level stakeholder interviews on the topic of response to outbreaks of yellow fever, case management, and the need for diagnostic testing. The responses have been synthesized to generate use case scenarios identifying specific cases in which a field test would be used. Additionally, target product profiles that include the characteristics required of field tests in order to address these identified use case scenarios have been created from the information collected during the interviews. These use case scenarios and target product profiles will be used to inform the research and development of field-deployable yellow fever tests and their introduction at the country level by operational organizations, leading to accelerated implementation. In parallel, the information will also be used to inform strategies for verification and

validation of such tests; begin to identify commercial viability, including market size, segmentation, and uptake; and introduction at the country level by operational organizations leading to accelerated implementation.

1208

QUESTIONS RAISED BY AN ANALYSIS OF VISCEROTROPIC REACTIONS TO THE YELLOW FEVER VACCINE

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The live virus yellow fever vaccine is integral to the control of yellow fever. Previously thought to be the safest of the live virus vaccines, it is now known to cause a rare but frequently serious and often fatal disease termed yellow fever vaccine-associated viscerotropic disease (YEL-AVD). Analysis of known cases has revealed that the vast majority can be assigned to risk groups: males ≥56, women in the prime child-bearing years, individuals thymectomized as treatment for thymoma, infants and children ≤11, women with systemic lupus erythematosis, and brothers with Addison's disease. In addition there was one case each with five other autoimmune diseases and one case whose older sister had died of YEL-AVD. Questions raised by the analysis include: 1) what is an appropriate estimate of the incidence? Although usually stated as 0.3 to 0.4 per 100,000 vaccinees, the data from individual studies vary from none in 2.6 million in Africa to 11.7 per 100,000 in Peru; 2) Do the variations in case fatality rate from 88% in South America to 41% in prospective travelers reflect differences in reporting or differences in vaccine safety (vaccine from South America is a derivative of 17DD and that used elsewhere of 17D-204); 3) Does the occurrence of three cases in young women in Peru reflect an unsuspected ethnic association possibly even related to a case in a young Spanish woman?; 4) Are there underlying genetic immune defects in each of the risk groups and is there a commonality in the defects such as a deficiency in thymic function or a defect in innate immunity; and 5) Does the concentration of fatal cases in women between the ages of 19 and 34 result from factors likely to be present in that age group? Investigation of these questions ranges from the simple ascertainment of the family history (known only in part for one of the young Peruvian women) to sequencing of the human genome. Answers are of concern not only to recommendations for the administration of yellow fever vaccine but also to the safety of vaccines under development that incorporate the yellow fever virus vaccine background.

1209

GENETIC DETERMINANTS ASSOCIATED WITH TICK AND MOSQUITO FLAVIVIRUS COMPETENCE

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Flaviviruses are positive-strand RNA viruses that infect more than 100 million people each year. While most flaviviruses exist in an arthropod vector (tick or mosquito)-vertebrate host life cycle, some flaviviruses have adapted to a single host life cycle in either an arthropod or a vertebrate host. The genetic determinants for flavivirus adaptation to a life cycle (dual host or single host) and whether the cycle type specificity constrains the virus's ability to adapt when subjected to new selective pressures has yet to be determined. Understanding the genetic determinants and adaptive constraints these factors impart on these flaviviruses will enhance our ability to design an attenuated, host-restricted, yet replication-competent vaccine candidate. Chimeric constructs between mosquito-vectored West Nile virus (WNV) and the tick-vectored virus deer tick virus (DTV) have been developed to identify the genetic elements that are responsible for host tropism and evaluate the versatility of these determinants in mammalian, tick, and mosquito cell types as well as an *in vivo* models for these systems. In mosquito and Vero cells, WNV parental and DTV-prME/WNV chimeric virus replication profiles did not differ and while the DTV parental and WNV-prME/DTV chimeric virus exhibited similar replication patterns. In ISE6 *Ixodes* tick cells, DTV reached a peak titer of 6.3 log10 PFU/ml, whereas the WNV-prME/DTV chimeric virus only reached a peak of 4.3 log10 PFU/ml. WNV and DTV-prME/WNV viruses were both poorly fit in tick cells, replicating to just above the level of detection. Our current findings indicate that for mosquito cells (C6/36) and mammalian cells (Veros), the nonstructural regions appear to be the dominant determinants of viral phenotype; however, in tick cells the determinants appear to reside in both the structural and nonstructural elements.

1210

EVALUATION OF THE T380R MUTATION IN THE RGD MOTIF OF YELLOW FEVER VIRUS ENVELOPE PROTEIN DOMAIN III IN *AEDES AEGYPTI*

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Glycosaminoglycans are ubiquitously expressed negatively charged macromolecules that have been found to support viral entry and dissemination in several arboviruses. The RGD motif is located in the envelope protein domain III (EDIII) of the mosquito-borne flaviviruses. This motif has been found significant in the binding interactions between virions and negatively charged molecules *in vitro* as well as viral dissemination and the associated viral pathogenesis in mammals. However, its importance in viral infection and dissemination in the mosquito vectors remains to be evaluated. Knowledge of this mechanism is critical for understanding the governance of vector competence of the mosquito-borne flaviviruses. As the prototypical flavivirus, the reverse genetics system of yellow fever virus (YFV) provides a unique platform to characterize and evaluate the individual genetic *loci* in the viral genome by the comparison of the virulent Asibi strain, which disseminates and infects mosquitoes, and the live-attenuated 17D vaccine strain, which does not have the capacity to disseminate attributable to mutations generated during the serial passage process. An *in vitro* transcribed viral RNA rescued mutant virus containing the T380R mutation located within the RGD motif in EDIII of YFV 17D strain was evaluated for infection and dissemination capability in *Aedes aegypti* mosquitoes infected per os. Female mosquitoes were collected and dissected on 7, 10 and 14 days post infection. The bodies and secondary tissues of these mosquitoes were homogenized and titrated to assess the efficiencies of viral infection, replication and dissemination. These titrations provided the knowledge to foster the identification of a genetic determinant for the vector competence and viral attenuation of YFV.

1211

PHENOTYPIC CHARACTERIZATION OF YELLOW FEVER VIRUS CONTAINING TWO POINT MUTATIONS IN THE ENVELOPE DOMAIN I AND DOMAIN II

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The limit infectivity and dissemination of the attenuated yellow fever virus (YFV) 17D vaccine strain in *Aedes aegypti* is attributed to mutations in the envelope protein. The five specific mutations in the envelope protein domain I (EDI) and domain II (EDII) have significantly reduced the infection rate among the engorged mosquitoes at 14 days post infection. Although the flavivirus EDI and EDII domains are not directly involved in host cell receptor binding, their role in supporting structural rearrangement and membrane fusion have been found essential for effective viral entry. Two

point mutations, G52R and T173I, were selected for non-conservative changes in the biochemical properties of amino acid substitutions and their proximities to the molecular hinge regions that accommodate the structural alterations generated during the viral entry. The objective of this study was to identify the genetic *loci* that reduced the viral infectivity of YFV Asibi strain amidst the serial passage process resulting in the attenuated YFV 17D vaccine strain. Mutations were introduced simultaneously and separately into the cDNA infectious clones of YFV to evaluate their infectivity and dissemination in *Ae. aegypti*. Mutant viruses were recovered from *in vitro* transcribed RNA and administered to *Ae. aegypti* per os in a bloodmeal. The viral infectivity, replication and dissemination capacities were evaluated by TCID50 titration of homogenized bodies, heads, wings and legs from mosquitoes collected on 7, 10 and 14 days post infection. Genetic stability of mutant viruses was also determined by nucleotide sequencing.

1212

WEST NILE VIRUS INFECTION INFLUENCES SYMBIOTIC BACTERIAL POPULATION MODULATIONS IN *CULEX PIPIENS*

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We are beginning to explore the natural microbial community that exists within insect vectors and the effects the mosquito microbiome is eliciting on vector-virus interactions and ultimately the transmission of arboviral pathogens. *Wolbachia,* a natural symbiotic bacterium of many mosquito species including *Culex pipiens*, has been the focus of many studies which have shown repeatedly that this bacterium has an effect on arboviral susceptibility, replication and transmission. It has been reported that *Wolbachia* limits West Nile Virus (WNV) susceptibility in *Cx. quinquefasciactus* and replication in *Aedes aegypti*, similar to studies with dengue virus, chikungunya virus and yellow fever virus, yet these negative correlations seemed to be bacterial and virus-strain dependent, suggesting complex interactions between symbiotic bacteria, host/vector and pathogen strain. Our results demonstrate a positive correlation between relative proportions of *Wolbachia* and WNV viral load in *Cx. pipiens*. Although *Wolbachia* has been the focus of most studies, it is likely that interactions with other bacterial species similarly have the capacity to modulate arboviral infections in mosquitoes. Our results suggest that the *Cx. pipiens* microbiome is being affected by *and* having an effect on WNV susceptibility in our colonized mosquitoes, where shifts in microbial populations are observed upon exposure alone to virus and are not predicted simply by infection status but, by WNV exposure in the absence of detectable infection. Specifically, our data demonstrate that many individual bacterial strains and/or species are associated with viral exposure and effects appear to be strain-specific and reveal novel correlations between the relative amount of Flavobacteria and Enterobacteria with WNV body titer.

1213

DEVELOPMENT OF IMMUNOHISTOCHEMICAL TECHNIQUES FOR DEFINING THE PATHOGENESIS OF WEST NILE VIRUS IN THE BRAIN OF EXPERIMENTALLY INFECTED HORSES

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West Nile virus (WNV) induces polioencephalomyelitis and results in the death of one-third of symptomatic horses and a 6% mortality rate in humans. This neurotropic virus elicits an immune response from both infiltrative, peripheral immune cells and resident glial cells. Perivascular cuffing and gliosis are commonly found pathologic changes in the basal ganglia, thalamus, brainstem, and ventral horn of the spinal cord in both species. To date, descriptive analysis of the inflammatory response in WNV infected brains is mostly of experimental disease in rodent models and not in the naturally occurring, outbred equine host. This study quantifies the cell populations found in the diencephalon and metencephalon of horses experimentally infected with WNV via intrathecal inoculation. Based on target antigen location and antibody specificity, immunohistochemical protocols were developed for the identification of resident and peripheral cells in the brain in formalin fixed paraffin embedded and snap-frozen, fresh tissues. Monoclonal and polyclonal antibodies, developed from a variety of species including human, swine, and equine, were used for identification of CD3+,CD4+, CD8+, and B lymphocytes, macrophages, astrocytes, microglia, and neurons. Early results demonstrate that CD3+ T-lymphocytes are the dominant infiltrative population with few macrophages/neutrophils and scarce CD 79+ B-cells. CD4+ and CD8+ populations are currently under investigation. Microgliosis was significantly higher in infected tissues as compared to control tissues, whereas astrocyte counts were not significantly different. Highly variable staining of neurons in both infected and non-infected horses was noted and is undergoing additional investigation. This study will provide new information in understanding the basic pathophysiology of WNV in the naturally occurring host and may bring new light to the role of uncommonly studied cell types like microglia.

1214

WOLBACHIA DOES NOT INHIBIT WEST NILE VIRUS (WNV) IN THE MOSQUITO *CULEX TARSALIS*

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Novel strategies are required to control mosquitoes and the pathogens they transmit. One attractive approach involves the maternally inherited endosymbiotic bacterium, *Wolbachia*. After artificial infection with *Wolbachia*, many mosquitoes become refractory to infection and transmission with diverse pathogens. By manipulating arthropod reproduction, *Wolbachia* can spread throughout the host population, replacing the natural vector population with one that cannot maintain pathogen transmission cycles. We evaluated the effects of *Wolbachia* (*w*AlbB strain) on infection, dissemination and transmission of West Nile virus (WNV) in the mosquito *Culex tarsalis*. After inoculation into adult female mosquitoes, *Wolbachia* reached high titers and disseminated widely to numerous tissues including the head, proboscis, ovarian follicles, thoracic flight muscles, and fat body. Contrary to other systems, *Wolbachia* did not inhibit WNV in this mosquito. Rather, in the majority of replicates, WNV infection rate was significantly higher in *Wolbachia*-infected mosquitoes compared to controls. This is the first observation of *Wolbachia*-induced enhancement of a human pathogen in mosquitoes, suggesting that caution should be applied before using *Wolbachia* as part of a vector-borne disease control program.

1215

EVOLUTION OF WEST NILE FEVER IN EUROPE: CLOSE MONITORING OF THE DISEASE SINCE 2011

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West Nile fever is a viral disease transmitted by mosquitoes that affects birds, equids and humans. Humans usually present asymptomatic infection or mild disease but neuro-invasive infection occurs. Crucially the virus can be transmitted through blood transfusion or organ transplants. The disease has been known in Europe for more than 50 years but it particularly re-emerged in Romania in 1996. Since 2004, two lineages have been co-circulating in the European Union. And since 2010, a geographical

expansion has been observed. To appraise the evolution of the disease, ECDC monitors human cases in Europe and neighbouring countries using epidemic intelligence methods. Since 2011, information concerning human cases from 63 countries of the WHO European region and the Mediterranean basin is collected through the West Nile mapping tool and publicly displayed on ECDC website, on a weekly basis, during the transmission season (June to November). Comparison of results obtained in 2011 and 2012 shows that, in 2012, the first cases were reported much earlier, the extension of the affected zone was broader and the density of areas reporting cases was higher. Hence, the total number of cases was higher in 2012 in the European Union (237 vs. 124) and neighbouring countries (670 vs. 203). In addition, several countries reported cases for the first time. Comparison of the geographic extension since 2010 shows that new areas were affected each year. In the evolution of the disease since 2010, the respective role of enhanced surveillance and change in the epidemiology of West Nile fever is discussed. On one hand, surveillance activities have been reinforced, i.e. awareness of health professionals is higher, special/active surveillance activities have been implemented. On the other hand, the epidemiology of West Nile fever has also changed, two lineages co-circulate in many countries and new strains are detected. In addition, some new questions are raised, e. g. the role of multiple introductions of the virus, the relations between animal and human cases, the impact of the climate.

1216

GENETIC ANALYSIS OF WEST NILE VIRUS HUMAN ISOLATES FROM THE U.S. SHOWS STEADILY INCREASING VARIABILITY FROM 2002 - 2012

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Since introduction into the U.S. in 1999, West Nile virus (WNV) has become endemic, causing annual outbreaks for 14 consecutive years. The rapid spread of WNV and pattern of recurring outbreaks in the US differs from the sporadic outbreaks previously observed in the old world, suggesting a potential role for viral adaptation to domestic vectors and hosts. Viral adaptation through genetic mutations has the potential to alter viral growth characteristics and virulence. Most (80%) human infections with WNV are asymptomatic; of the symptomatic cases, <1% are neuroinvasive infections (1:150 to 1:350 total infections) which have a high mortality rate (14%). Over the past 14 years WNV has caused ~37,000 human cases of serious illnesses including 15,975 neuroinvasive cases and 1,505 deaths reported to the CDC, with intense outbreaks of human cases in 2002 (4,156 cases) 2003 (9,862), 2006 (4,269) and 2012 (5,387). We investigated the degree of genetic variation in human infection by sequencing 78 (47 full and 31 partial) WNV isolates produced from human plasma, obtained from different geographical locations of the U.S. from 2002-2012. Compared to the sequence of the ancestor strain WNV-NY99, results showed increasing genetic variability over time including deletions and insertions in the 3'UTR. Most mutations were silent transitions, and the number of nucleotide mutations ranged from 20 to 81 resulting in 3 to 17 amino acid substitutions. Our study shows that over 14 years in the U.S., the WNV genome varied at a slow but steady rate when compared with WNV-NY99; increasing from 0.18% diversity in 2002 to 0.54% in 2009 and 0.65% in 2012. The mean nucleotide substitution rate for our WNV isolates through 2011 was 5.06×10^{-4} substitutions/site/year. Genetic surveillance is essential for public health since mutations could potentially affect viral pathogenesis, decrease performance of diagnostic assays, and negatively impact efficacy of vaccines and development of specific therapies.

1217

DEVELOPMENT OF A MICROARRAY-BASED ASSAY TO PERFORM MOLECULAR EPIDEMIOLOGICAL SURVEILLANCE OF WEST NILE VIRUS

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Since its first detection in the U.S. in 1999, West Nile virus (WNV) has become endemic in the Western Hemisphere. WNV is primarily transmitted by mosquito bites, but can also be transmitted by blood transfusion. WNV is estimated to have infected ~4 million humans in the US causing >36,000 cases of severe disease and 1,505 deaths reported to CDC. The speed of WNV's spread raised great concern and prompted a detailed investigation of the genetic evolution of WNV in search of causes for its rapid adaptability. Of concern is the potential for mutations in the WNV genome to affect the performance of diagnostic and screening assays, viral pathogenesis and therapeutic approaches. Thus, the detection of variants that may appear in the course of WNV outbreaks is extremely important and can only be achieved by the genomic characterization of new WNV isolates in a timely fashion. To address this need, we have developed a microarray-based assay capable of direct analysis of genetic variation in field specimens. The assay detects mutations by hybridizing biotinylated samples to oligoprobes covalently immobilized on slides surface covering the target region of the WNV genome with subsequent silver staining. Assay validation was performed using previously sequenced WNV isolates from the US, 2002-2012. The new assay detected unambiguously all mutations previously identified by traditional sequencing analysis. In addition the new assay eliminated the need for viral isolation by tissue culture, because viral RNA is isolated directly from plasma using magnetic beads loaded with specific oligoprobes. The identified mutants can be further sequenced using classical Sanger or next generation sequencing methods to determine position and character of mutations. The use of the described microarray for an initial screening of WNV isolates can notably reduce the time of analyses of circulating genetic variants and sequencing related expenses, and can be used as a valuable tool for rapid epidemiological surveillance of WNV during annual outbreaks.

1218

CHARACTERIZATION OF WEST NILE VIRUS-INDUCED MEMBRANE STRUCTURES REVEALS A NOVEL ROLE FOR NS1 PROTEIN IN INDUCTION OF VESICLE PACKETS FOR VIRAL RNA SYNTHESIS

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West Nile Virus (WNV) modifies intracellular membranes of the host cell, resulting in the formation of clusters of vesicle packets (VP) to establish its sites of replication. Although the role of VP for viral RNA synthesis is well documented, the WNV protein(s) responsible for the induction of the VP has not yet been identified. In this study, we describe a novel role for NS1 in the induction of these VP structures during WNV infection. We infected HEK293T cells with the WNV New York 1999 strain (NY99) and analyzed the localization of the NS1 protein in VP structures over time using immunofluorescence (IF) assay. WNV replication was determined using qRT-PCR and plaque assays, and NS1 protein expression was verified using Western Blot. To demonstrate the induction of VP with only NS1 protein, we synthesized cDNA from WNV NY99 RNA using reverse transcriptase and amplified the entire NS1 gene, including the endogenous signal sequence from the WNV envelope structural protein, by high-fidelity PCR. To visualize expressed NS1 in transfected cells, green fluorescent protein (GFP) or V5 epitope harbored in the expression vector was fused to the C-terminal end of the NS1 gene. Plasmids were transformed into

chemically competent *E. coli* (DH5α) cells and were isolated, purified and sequenced. The resulting plasmids were used for expression of NS1 protein in transfected HEK293T cells. The VP structures induced by only NS1 were analyzed using transmission electron microscopy (TEM). Using high-resolution confocal microscopy, NS1 protein was found to localize to VP, which appeared as fluorescent particles (FPs) scattered in the cytoplasm along with viral replicating RNA. Toward the end of the eclipse phase of infection, aggregation of FPs at the perinuclear region coincided with increases in viral RNA copies and virion production. TEM data using NS1 transfected cells revealed that the NS1-induced membrane structures were similar to VP structures observed in infected cells. NS1 has been implicated in the viral RNA replication and results from this study suggest that NS1 plays a role in VP induction. Studies are underway to explore the sequential events in VP biogenesis, and determine whether any host proteins are involved in VP formation and expansion.

1219

RARE NEUROLOGIC DISORDERS IN HIV POSITIVE CHILDREN TREATED WITH LONGTIME HAART

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Neurological manifestation of AIDS is of at least different etiology. The aim of this short communication is to describe neurologic disorders due to either HIV-related opportunistic coinfection or drug related toxicities in children with AIDS from Cambodia treated 10 years with highly active antiretroviral treatment (HAART). One hundred thirty seven (137) children (age 2-19 years) treated with HAART since 2003 (6-10 years follow up) have been analyzed concerning opportunistic infection, other coinfection, renal, hematologic parameters, X- chest ray, received of antiretrovirals (HAART), antituberculotics and antibiotics. We recorded prospectively all data since onset of HAART in 2003 - 2005 up to December 2012. Incidence of drug related neuritis due to stavudine (HAART) was less than 1% and have due to anti-TB agents accompanying HAART in HIV and tuberculosis coinfected patients. Coinfections with neurotropic viruses were higher: 1 case CMV retinitis, 18 herpes zoster neuritis and 37 generalized HZV infections (varicella) after onset HAART in children were recorded. All children responded well to antivirotic therapy with ganciclovir (CMV), vanciclovir or acyclovir (HZV) in combination with first line HAART. Neurologic disorders in children with HAART are less frequent than in adults. Antiretovirals and antituberculotic seems to be safe and less hemotoxic as reported in children then adults. Coinfections with neurotropic viruses were higher. All children responded well to antivirotic therapy with ganciclovir (CMV), vanciclovir or acyclovir (HZV) in combination with first line HAART.

1220

CHALLENGES OF FINDING HIV PATIENTS WHO HAVE DROPPED OUT OF CARE AFTER DIAGNOSIS AT AN HIV REFERENCE SERVICE IN LIMA, PERU

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In low-resource settings, HIV programs have difficulties finding patients who have dropped out of care, resulting in increased morbidity and HIV transmission. Stigma, discrimination and access to the clinic are main reasons for this problem. However, there is not information available about the status of patients who dropped out in Lima (Peru) to identify these patients and prepare an adequate intervention. This study has the aim to determine the status of HIV patients currently out of care at the HIV Clinic at Hospital Nacional Cayetano Heredia in Lima, Peru since HAART program was established. Among 3085 enrolled patients between 2004-2010, we identified 753 adult patients whose last medical care visit at the HIV Service occurred at least 12 months before March 2011. Patients with phone numbers were called first and home visits were attempted for patients who could not be reached by phone. This contact data was registered at enrollment into HIV Clinic. No exclusion criteria were used. The mean age among the 753 patients was 37.97 (SD: 11.5) and 475 (63.1%) were male. The median CD4 at enrollment was 246.5 (IQR: 354). 134 (17.8%) were located, 29.9% (40/134) of them were dead. 434 (57.6%) were not located for the following reasons: false address (18.9%), didn't live in the address provided (19.1%), hard to find the address (13.8%), the patient move and don't provide new address (17.3%) and non phone answer (30.9%). 185 (24.6%) were not approached for having incomplete data (31.4%) or for living outside hospital`s area of influence (68.6%). Non-located patients were not different in sex (64.94% vs. 54.47% males), age (38.02 years vs. 37.7 years; p-value 0.79) and CD count (238.5 vs. 257; p-value: 0.58) compared with located patients. Nearly 82% of HIV patients who dropped out of care cannot be found, and these patients are not different than located patients. Improving retention in care requires addressing the reported barriers and implementing a follow-up system to verify and update patient contact information until they can be returned to care.

1221

METHODOLOGICAL FLAWS IN THE HIV-STI TRIALS

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Substantial evidence indicates that sexually transmitted infections (STIs) promote HIV transmission by producing genital ulcers, inflammation, and viral shedding. The burden of untreated STIs is far higher in sub-Saharan Africa (SSA) than in any other region. Ten randomized controlled trials in SSA examined effects of STI control on HIV incidence. Only the first trial (Mwanza) produced statistically significant results. Consequently, support for STI treatment for HIV prevention has faded. The 9 post-Mwanza trials suffer from insufficient exposure contrast because ethical considerations require extensive STI treatments for controls, leading to small differences in STI outcomes between arms. The trials tested alternative treatments of bacterial or viral STIs, not both, and were thus subject to confounding from STIs not considered in the trial design. Substantial treatments for controls affected sexual behavior and STI outcomes between arms. No trials examined genital infections other than STIs that could enhance HIV transmission or acquisition. Thus none of the trials addressed potential effect modification from biological interactions in genital microbial communities. None of the trials considered genital ulceration and inflammation from non-sexually transmitted pathogens, most importantly Schistosomiasis hematobium, which is highly prevalent in SSA. None considered ulcers infected with streptococci, staphylococci, or fungi. Treating one genital infection may have little effect on HIV incidence when other infections are untreated. All of the trials that estimated pre-trial HIV incidence found statistically significant decreases in HIV incidence among treatment subjects and controls taken together, suggesting that STI control programs can be effective in reducing HIV. Flaws in the design of the post-Mwanza trials render them unable to inform HIV-prevention policy. Given abundant evidence that STIs and other genital infections promote HIV, STI and other treatment should be considered an important method for reducing HIV incidence in SSA and elsewhere.

1222

FOLLOW-UP OF HIV-EPTB COINFECTION BY ULTRASOUND

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Extrapulmonary and disseminated TB coinfection in HIV patients mainly affects individuals in Sub-Saharan Africa but also immigrants living in Europe. The disease is difficult to diagnose and is most effectively treated when diagnosed early. Ultrasound can detect suggestive findings such as enlarged abdominal lymph nodes, pleural and peritoneal effusions and focal lesions in the liver and spleen. In some resource-poor settings these findings are relied upon to start empiric TB treatment. However, longitudinal changes of this ultrasound (US) pattern during TB treatment have not been investigated. In a retrospective study, 25 patients with culture confirmed HIV-EPTB co-infection were diagnosed and treated between 2005 and 2011 in our Hospital. Seventeen patients (mean age 36.4 , range 24-57, M/F 1.83 , 11 of them (64.7 %) african immigrants , mean CD4 149.11(range 5-576) had follow-up US (FASH protocol) at 1, 3, 6 and 12 months. In 7 patients (41,17 %) after 1 month of treatment, the FASH findings had completely disappeared, in 2 patients they had partially disappeared; all 9 patients successfully completed TB therapy. In the remaining 8 patients the US examination performed at 1 month showed an increased size of the previously detected lesions and/or the onset of new lesions: among them, three patients died during the follow-up (one while treating TB coinfection and two after completing TB treatment for AIDS-related conditions), one patient had MDR - TB and was lost to follow-up after different courses of therapy, three patients had a relapse, likely related to poor compliance; in one patient with a prolonged course of the infection the lesions disappeared only after two years of treatment. In conclusion, our experience suggests that early regression (after 1 month of therapy) of HIV-EPTB US signs is a good prognostic index for treatment in patients with HIV-EPTB. US protocols for HIV-EPTB co-infection should be validated in prospective studies as a potentially useful tool for the follow up of HIV-EPTB.

1223

CLINICAL AND LABORATORY CHARACTERISTICS OF DENGUE AND HIV CO-INFECTION: A MATCHED CASE-CONTROL STUDY

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Coinfection of dengue virus and human immunodeficiency virus (HIV) has been reported as case series with patients generally having mild manifestations even though they are immunocompromised. However, there is a lack of reported epidemiological study to support this hypothesis. In this retrospective hospital-based 1:4 matched case-control study, the clinical and laboratory profiles of ten Chinese HIV-infected dengue patients (cases) were compared with 40 Chinese HIV-noninfected dengue patients (controls), matched for age, gender, dengue diagnosis method, year of dengue presentation and hospitalization status. Univariate and multivariate conditional logistic regression were performed. Seven out of 10 cases had AIDS at the point of dengue presentation. The median period from HIV positive diagnosis to dengue presentation was 36.5 months with median CD4 counts of about 123 cells/mm3 six months before and after dengue presentation. A smaller proportion of cases (10%) was classified as DHF/DSS as compared to controls (52.5%;P=0.046). In contrast, a higher proportion of cases (50%) were classified as having severe dengue compared to controls (20%;P=0.05). The median fever duration was shorter in cases (3days) compared to controls (4days; P=0.032). However, the median hospitalization duration was longer in cases (10.5days) compared to controls (5days;P=0.054). There is no significant difference in supportive treatments even though the occurrence of tachycardia and shock were higher in cases (70%;90%) compared to controls (20%;35%) during hospitalisation. During presentation and hospitalisation, maximum pulse rate [adjusted conditional odds ratio (ACOR)= 1.31;95% confidence interval(CI)=1.02-1.25; ACOR=1.11;95%CI=1.01-1.22) and esinophils level (ACOR=2.82;95%CI=1.06-7.51; ACOR=1.90;95%CI=1.17-3.09) were positively associated with coinfected patients. HIV-infected dengue patients may clinically present as mild as HIV-noninfected dengue patients during presentation, but may not throughout the hospitalisation. Close observation and supportive treatments for HIV-infected patients coinfected with dengue remain critical.

1224

CROSS-CULTURAL EXEMPLARS ON HIV/AIDS RISK AND RESILIENCE AMONG YOUTH: THE PERSPECTIVE OF CHILD AND ADOLESCENTS FROM DIFFERENT BACKGROUNDS

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This study was undertaken to examine evidence available by considering the exemplars or indicators of categories in risk and resilience among youth as well as child abuse and neglect as it directly exposes them to risks of HIV/AIDS infection. Child abuse has become an international concern and has been discussed extensively in developed countries and regions, example of which is now being translated to the developing nations. A retrospective cohort study of children and youths that visited the clinics both in Nigeria and Malaysia by accessing the hospital medical records and data was identified from linked de-identified population level data. Results were analysed on the patterns and views as well as trends in prevalence of assault, maltreatment, risk and resilience, and risky sexual practices as a result leading to HIV/AIDS/STDs; which were further investigated. It is pertinent to note that - Youth and children's disclosure of abuse is often affected by the culture in which they live, like filial piety and loyalty to parents. Some of the views expressed by children, however, are very much akin to those of adults, such as the factors they would consider in deciding whether a case is child abuse or not. Youths do not have a homogeneous view on issues about the risk and resilience existing in child abuse and neglect, and their awareness and sensitivity to different kinds of child abuse are also different, also leading to varying levels of information about HIV/AIDS and sexual education. In conclusion, there has been steady increase in the prevalence of assault and maltreatment. In order to continue to develop appropriate services and policies for vulnerable youth, it is necessary to continue definitional clarity for research in child maltreatment, in tandem with parental and child characteristics which can provide one source of evidence-basis to meaningful child protection case classifications.

1225

CONTRIBUTION OF XPERT MTB/RIF ASSAY IN THE DIAGNOSIS OF TUBERCULOSIS AMONG PATIENTS LIVING WITH HIV IN DAKAR, SENEGAL

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Lack of a rapid, sensitive, and specific diagnostic test for tuberculosis (TB), especially in resource poor countries, greatly complicates TB control worldwide. A new diagnostic method based on real time PCR offers an advantage compared to microscopy, which has low sensitivity, and culture,

which has a long turnaround time. We performed a comparative study of diagnostic methods used in a TB endemic region on 128 patients with suspected TB. Sputum samples were analyzed by the Xpert MTB/RIF assay and results were compared to smear microscopy and culture. The study population of 128 patients aged between 18 to 74 included 96 men (75%) and 32 women (25%), of whom 52 patients (40%) tested HIV positive. 32 out of 35 smear and culture positive patients were detected positive by Xpert MTB/RIF assay resulting in 91.4% sensitivity in this group. Only 12 out of 20 patients with a negative smear and positive culture were positive by the assay. Among patients with negative smear and culture 6 out of 65 sputa were detected positive by the assay (9.2%), with one invalid result. All the six patients were HIV positive and naïve to antiretroviral treatment. The time to diagnosis was 2 hours for GeneXpert positive results versus one day for microscopy and 21days for culture. The GeneXpert assay performed well in our setting, with increased sensitivity over microscopy and short turnaround time. The GeneXpert positive sputa that were culture negative were likely true positives that were missed in culture. This comparison of methods available in Dakar shows the need for wider implementation of this new diagnostic test for tuberculosis infection, especially for improved care for people living with HIV.

1226

DRUG METABOLISM AND TRANSPORTER-BASED RATIONALE GUIDES REFORMULATION STRATEGIES TO REDUCE TREATMENT COSTS WITH TDF IN HIV/AIDS

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Tenofovir disoproxil fumarate (TDF) is a bisphosphonate ester pro-drug of tenofovir (TNF) designed to improve oral bioavailability. Nevertheless, bioavailability in humans remains below 30%. Improvements in bioavailability may allow a reduced dose and lead to significant cost savings in generic markets. TDF is subject to a two-step hydrolysis to first the mono-phosphonate ester (TNF-ME) and then to the active TNF. To prioritize reformulation options, drug metabolism and efflux transport were studied to identify targets that might increase the fraction absorbed (f_a). These included stability in simulated gastric (SGF) and intestinal (FaSSIF) fluids +/- pancreatic enzymes, restrictive absorptive permeability, efflux transport by P-glycoprotein (Pgp), and hydrolysis by intra-luminal and enterocytic esterases. TDF and TNF-ME were stable ($T_{½}$ >360 min at 37°C) in SGF and FaSSIF indicating protection from various pH conditions was unwarranted. However, both were unstable ($T_{½}$ <7.5 and 83 min, for TDF and TNF-ME, respectively) in FaSSIF supplemented with physiological levels of pancreatic enzymes. Permeability across MDCK-MDR1 cells demonstrated that TDF, but not TNF-ME or TNF was a substrate for Pgp efflux. Intrinsic permeability for TDF was modest (P_{appA-B}, 23 nm/s) but consistent with oral delivery. Permeability for TNF-ME and TNF was low (P_{appA-B}, <5nm/s) indicating TDF is preferred. Transport and metabolism studies in Caco2 monolayers demonstrated that esterase inhibition by excipients including propyl paraben (PP) increased f_a 8.5 fold (15.3% and 1.8% of dose delivered with PP or in control, respectively). Inhibition of Pgp by d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS) improved f_a 2 fold (3.5% of dose delivered). Combined esterase and Pgp inhibition increased f_a 10.9 fold (19.7% of dose delivered). In conclusion, *in vitro* studies suggest TNF bioavailability might be significantly improved by co-formulating TDF with excipients that inhibit Pgp efflux and esterase hydrolysis. Bioavailability studies are underway in dogs to evaluate this hypothesis.

1227

PREVALENCE AND BIO BEHAVIORAL CORRELATES OF CARCINOGENIC HPV INFECTION AMONG HIV INFECTED MEN WHO HAVE SEX WITH MEN (MSM) IN LIMA, PERU

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Individuals practicing unprotected anal intercourse are at high-risk for HPV infection and other STI. Currently, fifteen different types of HPV have been classified as of high-risk based in their carcinogenic potential, including anal cancer. Furthermore, STI may have an impact on HPV transmission and acquisition.In 2012 a bio behavioral survey was conducted among 152 adult HIV-positive MSM in Lima, Peru to assess the prevalence and correlates of carcinogenic HPV infection. Subjects underwent a structured behavioral interview and serology testing for HTLV and syphilis, urine testing for *Chlamydia trachomaties* and *Neisseria gonorrhoeae*; and rectal swabbing for HPV DNA testing by PCR. All participants considered themselves either "gay" (75.7%) or bisexual (24.3%); 50.9% reported having a versatile role in bed. The mean time from HIV diagnosis to enrollment was 4.4 years (SD 3.9 years); 78.3% were receiving HAART at the time of enrollment. Mean CD4+ cell count was 437.4 cells/mm^3 (SD 193.5). Eighty participants (52.6%) reported symptoms of STIs in the preceding six months. One hundred forty-seven participants (96.7%) had HPV infection; 90.5% of them with one or more carcinogenic type. The most prevalent types were 6, 16, and 58. The prevalence of urethral chlamydia (2.6%) and gonorrhea (0.7%) were very low. Syphilis seroreactivity was 26.3%. Factors associated with presence of carcinogenic types of HPV were ever having receptive anal intercourse (OR=10.17; 95% C.I. 2.12 - 52.61), having a versatile (OR=5.91; 95% C.I.1.36 - 25.63) or exclusively receptive role in bed (OR= 9.50; 95% C.I. 1.07 - 84.09). Given the high prevalence of carcinogenic types of HPV in the target population, different strategies are needed to improve compliance towards safer sex, including male adolescent HPV vaccination regardless HIV status.

1228

ARVDS ADHERENCE: PATIENTS SELF REPORTS, CD4 COUNTS VS ARVDS BLOOD CONCENTRATIONS

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Quite a number of researches and publications have addressed HIV infected patents' adherence to prescribed antiretroviral drugs (ARVDs). Adherence to ARVDs ensures adequate blood levels of these drugs with resultant control of HIV disease state and reduction of infectivity. In the regular clinical setting especially those found in resource- limited regions typified by Nigeria, patient self-reports, CD4 counts, viral load, pill counts and pharmacy dispensing data have been used to monitor adherence, however, all have their limitations and may not give an indication of ARVDs absorption. This is against the back drop that the ultimate concern in therapeutics is the knowledge of blood concentrations of ARVDs achieved. Venous blood samples of 10 consecutive HIV infected patients on Lamivudine(L), Zidovudine(Z) and Nevirapine(N) combination for at least 2 months were collected after they consented to be enrolled in the study. Eight(8) of the patients had reported 100% adherence, while 2 of them skipped a dose each 2 weeks prior to the test. These samples were assayed for concentrations of LZN and CD4 count using HPLC Adilent 1200 series and Partec Flow Cytometer. The blood concentrations of L varied from 4.411-17.382 µg/ml (C pss= 1.6µg/ml); Z was 0.2040-0.3825µg/ml (Cmin/Cmax=0.02/2.29 µg/ml); N varied from 1.683-9.944 µg/ml(Cmin/Cmax=3.73/5.74 µg/ml). CD4 counts varied from 227-705 cells/ µL. The patients' reports of adherence to medication was proved right by the observed blood concentrations of the ARVDs which in the case of L was

above Cpss, The concentration of Z was also suggestive of same since it was above Cmin. Though, concentration of N was less than Cmin in some patients, gross analysis also suggest drug adherence. CD4 counts appeared to indicate adherence to ARVDs since the values got correlate with that expected among these patients. Patients self- reports and CD4 counts appear to be useful indicators of ARVDs adherence among this group of patients.

1229

NOVEL TENOFOVIR DISOPROXYL FUMARATE (TDF) FORMULATION TO INCREASE BIOAVAILABILITY FOR TREATMENT OF HIV/AIDS IN EMERGING MARKETS

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In the context of funding constraints and the need for continued treatment scale-up for HIV, pursuing interventions with a high return on investment and potential to produce significant global savings is critical. The Clinton Health Access Initiative (CHAI) has initiated a program to develop a novel, lower-dose formulation of TDF. This program will determine whether a lower dose alternative to Viread® will provide the same pharmacokinetics in a novel formulation targeted to increase bioavailability of the active ingredient. In collaboration with major stakeholders during the Conference on Antiretroviral Dose Optimization (CADO) in June 2010, and with input from a range of key experts, including process and formulation chemists, clinical pharmacologists and researchers, ARVs were ranked with the goal of reducing the cost while maintaining the effectiveness of current ARV regimens and to consider additional opportunities for optimizing treatment through manufacturing, formulation and/or dosage forms for delivery in resource limited settings. Reformulation of TDF was identified as the lead candidate to pursue. As TDF reformulation represents a novel approach to optimization, establishing proof of concept (POC) could result in significant contribution to the scientific community guiding similar research efforts for other products. R&D efforts thus far have resulted in more than twenty prototype formulations that have been screened in rat and dog studies. Currently, additional R&D is being conducted in the dog model to identify lead formulations to move into clinical studies. It is anticipated that straightforward, Phase I bioequivalence studies will be required for approval and acceptance of the novel TDF formulation or other reformulated products going forward. A significant effort focused on proactive engagement with regulators, normative bodies, and local opinion leaders is included to ensure that the technology and products being developed will be accepted and adopted in the marketplace in a timely manner. This roll-out platform can also be leveraged to support the uptake of optimized products in the future.

1230

NON-COMPLIANCE DURING MASS AZITHROMYCIN DISTRIBUTION FOR TRACHOMA IN THE GAMBIA

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Untreated individuals in mass treatment programs for infectious diseases can limit the beneficial effects to the community by maintaining a reservoir of infection. Treatment receipt was recorded against the community census during three mass treatment rounds (baseline, year one, year two) as part of a cluster randomised trial, in 48 communities in four districts in The Gambia. Factors associated with non-compliance were investigated in 1-9 year olds using random effects logistic regression. Two types of non-compliers were identified; present during the treatment team visit but not treated (PNT) and eligible for treatment but absent during the visit (EBA). At baseline 99 (1.0%) of 9790 children were PNT and 505 (5.2%) EBA. Non-complier types differed by district (p=0.001) and household size (p<0.001). PNTs were generally from small households in medium to large EA within close proximity to water and with no TF diagnosis in a 0-5 year old in the household prior to treatment at baseline. Regression analyses compared PNTs and EBAs to treated children separately. At the year one and year two treatment rounds, being PNT was more likely for children previously PNT. At baseline, odds of being EBA were higher for children under six years old and for children who had not been examined for trachoma prior to treatment. EBA status was also associated with household level factors. Increased odds of being EBA at year one were seen for EBAs at baseline (OR=4.01; 95% CI:2.43-6.62). Increased odds of being EBA at year two was also noted for baseline EBAs and year one EBAs (OR=1.80; 1.10-2.96 and OR=1.57; 1.17-2.11 respectively). Non-compliance in The Gambia was low overall suggesting positive attitudes towards community-wide treatment. Results showing associations with household level measures and persistent non-compliance lends support to the hypothesis of household level clustering of, and possibly decision making regarding, compliance. Sensitisation of all household heads within communities ahead of treatment team visits could improve compliance amongst the target population.

1231

A MULTI-STAGE CLUSTER SAMPLING METHOD FOR MONITORING AND EVALUATION OF THE TRACHOMA CONTROL PROGRAMS IN THE TANZANIA

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The World Health Organization recommends control of trachoma through surgery, antibiotics, facial cleanliness and environmental improvement (SAFE) Strategy. Baseline survey of trachoma must be undertaken before SAFE implementation and impact surveys are recommended after 3-5 years of SAFE implementation. A multi-stage cluster random sampling design was adopted for surveys of trachoma in 2012. The survey had two components. The first was a baseline survey to estimate prevalence of trachoma and its associated risk factors in 10 districts considered at high risk for trachoma. Twenty (20) villages (clusters) were randomly selectedin each of the baseline survey districts. The second was impact survey of prevalence of trachoma and its associated risk factors in 3 districts following interventions with SAFE from 2005 to2011. In each impact survey district, villages were grouped into three or four sub-districts, configured to comprise at least two Wards thus permitting finer stratification depending on the number of Wards available. Ten villages were selected randomly from each sub-district, giving a total of 30 or 40 villages per district. For both surveys, villages were stratified by Wards with the aim at having equal representation of geographical administrative areas. The total population and households per Ward were used in the process of determining the number of primary sampling unit (village) required for each Ward. At least one village was randomly selected per Ward. Random sampling was used to select the number of villages required for each Ward. In the second stage households required to meet the desired sample were randomly selected in each baseline survey village (36) impact survey village (73). The households were equally allocated to the number of hamlets in each sampled village. In each hamlet, systematic random sampling was used to select the number of household to be surveyed. This survey design has provided the trachoma control programme in Tanzania a reliable method for estimating prevalence of trachoma for programmatic decision making.

1232

INTEGRATED MONITORING AND EVALUATION FOR LYMPHATIC FILARIASIS AND OTHER NEGLECTED TROPICAL DISEASES USING A MULTIPLEX BEAD ASSAY

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Transmission Assessment Surveys (TAS) are conducted to evaluate Mass Drug Administration (MDA) programs for lymphatic filariasis (LF) to determine when the level of incident infection in six to seven year old children is sufficiently low that population drug treatment may be ceased. After MDA is stopped, current WHO recommendations for surveillance are based on repeat TAS, using ICT to detect filarial antigen. We used a multiplex immunoassay that detects antibodies against multiple antigens as an integrated surveillance tool to assay blood spots from 1590 children who participated in a TAS for LF in the Dafra, Karangasso-Vigue, and Lena health districts of Burkina Faso. The multiplex assay is a flexible platform, which provides a facile tool to combine monitoring and evaluation opportunities among multiple NTDs. Using the multiplex antibody assay, LF seroprevalence as measured by detection of Bm14 antibody was 2.7%, compared to antigenemia prevalence by ICT of 0.3%. Although the primary goal of the TAS was to estimate active transmission levels of LF, samples also were tested for antibody against antigens for onchocerciasis, strongyloidiasis and trachoma. In the Burkina Faso evaluation unit, the prevalence of antibodies against Ov16 (onchocerciasis) was 0.3%, the prevalence of antibodies against NIE (strongyloidiasis) was 1.4% and the prevalence of antibodies against trachoma antigens was 2.9% as measured by antibodies against Pgp3 antigen and 3.4% as measured by antibodies against CT694 antigen. The use of the antibody multiplex assay in this context provides a proof of principle of the potential utility of integrated TAS for programmatic monitoring and evaluation for NTDs. With this technology, it is also possible to coordinate NTD monitoring with efforts to evaluate the impact of malaria inventions, vaccine coverage or the health impacts of water and sanitation projects.

1233

UPDATES ON TRACHOMA PREVALENCE IN TANZANIA AS PER 2012 BASELINE SURVEY

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Control of blinding trachoma through the surgery, antibiotics, facial cleanliness and environmental improvement (SAFE strategy) is a global initiative that was endorsed by the World Health Organization in 1998. The SAFE strategy utilises surgery for trachomatous trichiasis (TT), antibiotics for mass treatment of active trachoma, and facial hygiene and environmental improvement to reduce transmission of ocular chlamydia. Prior to SAFE implementation, a baseline survey on trachoma prevalence must be undertaken. This survey primarily assists programmes to understand the epidemiology of the disease in order to deliver appropriate treatment and control interventions. As of 2012, only 50 out of 130 districts had been mapped countrywide in a phased approach in 2004 and 2006. In the 2012, another phase of trachoma mapping baseline surveys was done to determine the magnitude of trachoma endemicity and its associated risk factors in the un-surveyed at risk districts of Mbulu, Serengeti, Bariadi, Uyui, Urambo, Karatu, Babati, Arumeru, and Korogwe. A multi-stage cluster random survey sampling was applied whereby 20 villages (clusters) and 36 households per cluster were surveyed. A total of 6,777 households were surveyed and 29,230 participants (93.2% of those enumerated) examined for trachoma signs. Of the 11,417 children aged 1-9 years examined, overall prevalence of trachomatous inflammation-intense (TF) was 1.3% (95% confidence interval [CI] 1.0-1.7). Prevalence of TF varied by district ranging from 0% in Urambo to 2.9% in both Mbulu and Karatu. The proportion of children with a clean face (defined as absence of ocular or nasal discharge) was 84.4%. A total of 17,813 people aged 15 years and above were examined of TT. Overall prevalence of TT was 0.4% (95% CI 0.2-0.6) and varied by district ranging from 0.1% in Bariadi and Urambo to 0.7% in Babati. Based on the survey findings none of the surveyed districts qualify for implementation of the SAFE strategy.

1234

AN ANALYTICAL REVIEW OF ACADEMIC, TECHNICAL AND LABORATORY CAPACITY FOR NEGLECTED TROPICAL DISEASES IN NIGERIA

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Nigeria has a significant burden of the neglected tropical diseases (NTDs) that are currently being targeted for control and elimination globally through the preventive chemotherapy strategy. Four main helminth infections, onchocerciasis, lymphatic filariasis (LF), schistosomiasis and soil transmitted helminthiasis (STH) are specifically being targeted with the mass administration of anthelminthic medicines. Nigeria is preparing to scale up its efforts to control and eliminate these NTDs and will require a range of internal technical support and expertise for mapping, monitoring and evaluating, operational research and documenting its success. This study aimed to examine the existing capacity on NTDs in academic and research institutes, and to determine how they may potentially support programmatic activities in Nigeria. Based on published data in the last five years from scientific literature and national institutional reports available in the public domain, all references on studies were systematically compiled into a database recording the institution, department, disease, research type (i.e. molecular, parasitological, immunological, epidemiological and/or entomological), international collaborator institute, journal name and impact factor. All institution locations were mapped, and the filarial disease loiasis also included given its importance to onchocerciasis and LF programmes. A total of 178 publications from 119 journals, highlighted that 47 institutions were involved in NTD research; of these 30 (64%) worked on schistosomiasis, 25 (53%) on onchocerciasis, 25 (53%) on LF, 22 (47%) on STH and 11 (23.4%) on loiasis. The most common type of research was parasitological, and the least was molecular studies. International collaborators were from a few selected institutes in Europe and USA, and publications were generally in low impact journals. This study shows that many institutions are working on NTDs in Nigeria and their capacity could be readily enhanced with training and resources to boost their skills, and increase their range of technical activities and research visibility, which will also help to provide essential technical and laboratory support to the national NTD programmes.

1235

THE ROAD TO TRACHOMA ELIMINATION IS NOT TRAVELED ALONE: SHARED BEST PRACTICES ACROSS MALI, NIGER AND TANZANIA IN DELIVERY OF TRICHIASIS SURGICAL SERVICES FROM 2008-2012

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Mali, Niger, and Tanzania - countries with a history of immensely high trachoma disease burden - have made substantial achievements over the past five years in their trachoma elimination efforts. Although all three countries are in various stages of scaling-up trichiasis surgical services, they have shared similar challenges and lessons learned. A systematic review of monthly program data (2008-2012) reported to Helen Keller International from Mali, Niger, and Tanzania was conducted to identify the fundamental lessons learned and challenges. From these, four themes in best practices were identified: "Leadership and Ownership," "Mobilization," "Quantity with Quality," and "Innovation for Improvement." Leadership and Ownership addressed the necessity of having national-, regional-, and district-level eye heath leaders fully engaged in an intensive leadership role for all aspects of surgical delivery. The direction provided by these individuals and the ownership they assumed served as a catalyst for the highest quality planning, implementation, supervision, and monitoring/evaluation of activities. Mobilization applied to the timely employment of human resources, such as trichiasis surgeons and supervisors; monetary resources to support consumables, equipment, and fuel; trusted community leaders, such as village chiefs and religious figures, to endorse the activities and encourage community participation; and health messaging through community radios, women's groups, and town criers to inform the public about trichiasis camps. Quantity with Quality went hand-in-hand; achieving district-level surgical goals required camps to be strategically planned to maximize output and resources, while simultaneously, quality control measures decreased the probability of post-operative trichiasis. Innovation for Improvement allowed existing strategies to be improved and perfected, with the development of new strategies to overcome evolving challenges. Implemented concurrently, these best practices will continue to guide each country in their efforts to eliminate blinding trachoma.

1236

ASSESSING DATA QUALITY OF NEGLECTED TROPICAL DISEASES PROGRAMS IN UGANDA

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High coverage is essential for the success of preventive chemotherapy (PC) programs targeting NTDs, and drug distribution data is routinely reported. However, national NTD programs have only rarely conducted formal data quality assessments (DQA) to evaluate the quality of reported data and data management systems, despite the widespread use of DQA for other public health interventions. The Uganda National NTD Program, collaborating with RTI-ENVISION and WHO, conducted its first DQA for NTDs at 16 service delivery points (SDP) in 4 sub-counties of 4 districts treated through PC in 2012. This DQA consisted of verifying available reported results through comparison with recounted values for 5 indicators at village, sub-county, and district; as well as interviewing individuals involved in PC data compilation and reporting to qualitatively assess the PC data management system. We discovered that reports were available 75% of the time, and 87% of reported results were verified at the district level. The interviews with community medicine distributors and their supervisors revealed somewhat limited understanding how to complete reporting forms at SDP and sub-county levels, which sometimes resulted in inaccurate reported results at these lower levels (range of average DQA data verification factor: 0.6-2). Standard tools for reporting were identified and fairly consistently used at the various levels; we found that in most cases, guidance was provided on how to complete tools, indicators to be reported, and to whom reports should be submitted, although many respondents were not informed when reports should be submitted. These findings suggest that the quality of the monitoring system is satisfactory, although additional training and supportive supervision for data compilation is needed to ensure accuracy of reported results. In the future, the national NTD program in Uganda, with support from partners, should strengthen data management training and supportive supervision; establish clear timelines for reporting; and develop job aides to facilitate data management and use.

1237

COORDINATED ASSESSMENT OF SOIL-TRANSMITTED HELMINTHIASIS AND LYMPHATIC FILARIASIS THROUGH TRANSMISSION ASSESSMENT SURVEYS IN BENIN AND TONGA

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Mass drug administration (MDA) for lymphatic filariasis (LF) programs has delivered over 2 billion treatments of albendazole plus either ivermectin or DEC, including many to communities co-endemic for soil-transmitted helminthiasis (STH), resulting in considerable reduction of the prevalence of both diseases. A transmission assessment survey (TAS) using lot quality assurance sampling, designed to determine if MDA for LF can be stopped in an evaluation unit (EU) after five rounds of annual treatment, may provide a valuable opportunity to integrate assessment activities by evaluating the impact of MDA on STH through a coordinated survey approach. Pilot studies conducted in Benin and Tonga assessed the feasibility of combining an STH survey alongside a TAS. Of the 30 schools (clusters) selected for a TAS in each EU, a subset of 5 schools per STH ecological zone was randomly selected, according to World Health Organization (WHO) guidelines, for the coordinated survey. In Benin, a total of 519 children were sampled in 5 schools and 22 (4.2%) were found positive for STH infection (*Ascaris lumbricoides, Trichura trichiura,* or hookworm) measured by the Kato-Katz test. All positive cases were classified as light intensity under WHO criteria. STH infection in children 6-7 years old (recommended by TAS) was not significantly different from those 8- 9 years old (recommended for STH by WHO guidelines). In Tonga, 10 of 30 schools were chosen for the coordinated TAS and STH survey resulting in 114 of 552 (20.7%) positive cases. All children sampled were aged 6-7 and all infections were of light intensity with the exception of one moderate *T. trichiura* case. Synchronous assessment of STH with TAS provides a convenient, well-timed assessment of infection prevalence to determine ongoing treatment decisions at the time MDA for LF may be stopped. The field experiences in both Benin and Tonga also highlighted potential time and cost savings through a coordinated approach. Refinement of a coordinated TAS and STH sampling strategy should be pursued in addition to further testing of alternate diagnostics to Kato-Katz for improved survey logistics.

1238

AFRICAN PROGRAM FOR ONCHOCERCIASIS CONTROL 1995-2010: IMPACT OF ANNUAL IVERMECTIN MASS TREATMENT ON OFF-TARGET NEGLECTED TROPICAL DISEASES

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Since its initiation in 1995, the African Program for Onchocerciasis Control (APOC) has had a substantial impact on the prevalence and burden of onchocerciasis through annual ivermectin mass treatment. Ivermectin is a broad spectrum anti-parasitic agent which also has an impact on other co-endemic parasitic infections. In this study, we assessed the additional impact of APOC activities on the burden of such off-target diseases. We first reviewed the literature to identify off-target infections that are likely to be impacted based on their endemicity in APOC countries and ivermectin efficacy against such infections, if any. Based on this review, we selected soil-transmitted helminthiases (STH; ascariasis, trichuriasis, hookworm, and strongyloides), lymphatic filariasis (LF), and epidermal parasitic skin diseases (EPSDs) as the most important, potentially impacted off-target infections. Using data on the number of given treatments and the latest estimates of the burden of disease, we calculated the impact of APOC activities on off-target infections in terms of disability adjusted life years (DALYs) averted. We estimated that between 1995 and 2010, annual ivermectin mass treatment has cumulatively averted about 500 thousand DALYs from co-endemic STH infections, LF, and EPSDs. This impact comprised an additional 5.5% relative to the total burden averted from onchocerciasis (9 million DALYs), and suggests that the overall cost-effectiveness of APOC is even higher than previously estimated.

1239

HEALTH-EDUCATION PACKAGE TO PREVENT WORM INFECTIONS IN CHINESE SCHOOLCHILDREN

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A third of the global population, mainly in developing countries, is infected with soil-transmitted helminths (STHs). Infection with these intestinal parasitic worms is associated with poverty in rural locations, inadequate sanitation and waste disposal, a lack of clean water, and poor hygiene and is common in areas with limited access to health care and preventive measures. Major success in preventing STH infections in Chinese schoolchildren as the result of a health education package we implemented incorporating a cartoon video. The study, undertaken in Hunan Province, showed a 50% efficacy in preventing the incidence of STH infection after a cluster randomized controlled trial in 38 schools involving 1718 schoolchildren, as previously published. The intervention proved highly successful with a significant impact evident across all outcome measures. We are now evaluating the efficacy of the educational package in two additional cluster-randomized controlled trials in China and in the Philippines. In China, we evaluate the package in a high STH prevalence setting in Yunnan Province. For the Philippines, the educational package will be culturally adapted so as to assess its efficacy in another Southeast Asian setting. The overall research objectives, study design and the main results of the Hunan Province trial will be described briefly and selected scenes of the animated narrative cartoon video, which forms the basis of the education package, will be shown. New STH control strategies are urgently needed, since current control efforts focusing on mass drug administration have been shown to be unsustainable due to rapid reinfection. The video-based educational package we have developed and trialled successfully provides a promising new tool for integrated STH control.

1240

THE MODELLING OF SCHISTOSOMIASIS ELIMINATION

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In the last few years momentum has shifted from a target of controlling schistosomiasis morbidity to aiming for possible elimination. However, there is currently an insufficient understanding of schistosomiasis transmission dynamics and the combination of interventions that may be required to reach elimination (such as mass drug administration (MDA), water, sanitation, and hygiene improvements (WASH), and snail control). This talk will describe the work that that the Schistosomiasis Control Initiative (SCI) and its London Centre for Neglected Tropical Disease Research (LCNTDR) partners is undertaking on this area, as part of a new operational research grant. A mathematical model of transmission dynamics for helminths is being developed in a related project within the LCNTDR, headed by Prof. Sir Roy Anderson. This is being constructed specifically for soil-transmitted helminths but the same underlying model will be utilised and extended to explore schistosomiasis transmission. Currently available datasets (those of the SCI others) will be used to estimate schistosomiasis-specific transmission and infection parameters for the model. Parameters will also be estimated using incoming data from trials on MDA, WASH, and snail control, as well as using figures taken from the published literature. It is hoped that the outputs of the modelling objective will help identify: the transmission breakpoint of infection in a population; the contribution of young children to transmission and the optimum approach for them; and the suggested schistosomiasis elimination strategies for different geographies and populations. It is also hoped that the outputs will be used to directly inform guidelines for the helminth control programmes.

1241

METALLOPROTEINASES 2 AND 9 ARE DIFFERENTIALLY EXPRESSED IN PATIENTS WITH INDETERMINATE AND CARDIAC CLINICAL FORMS OF CHAGAS DISEASE

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Dilated chronic cardiomyopathy (DCC) from Chagas disease is associated with myocardial remodeling and interstitial fibrosis, resulting in extracellular matrix (ECM) changes. In this study, we characterized for the first time, the serum MMPs 2 and 9 levels, their enzymatic activities as well as their main cell sources in peripheral blood from patients presenting the indeterminate (IND) or cardiac (CARD) clinical forms of Chagas disease. Our results showed that serum levels and enzymatic activity of MMP-9 are associated with the severity of Chagas disease. The analysis of MMPs production by T lymphocytes showed that $CD8^+$ T cells are the main source of both MMP-2 and MMP-9 molecules. Using a new 3-dimensional model of fibrosis we observed that serum from patients with Chagas disease induced an increase in the extracellular matrix components in cardiac spheroids. Furthermore, MMP-2 and MMP-9 showed different correlation

with matrix proteins and inflammatory cytokines in patients with Chagas disease. Our results suggest that MMP-2 and MMP-9 show distinct activities in Chagas disease pathogenesis. While MMP-9 seems to be involved with the inflammation and cardiac remodeling of Chagas disease, MMP-2 does not correlate with inflammatory molecules.

1242

CHARACTERIZATION OF INFECTIVITY AND IMMUNOGENESIS OF GENETICALLY ALTERED LIVE-ATTENUATED *LEISHMANIA DONOVANI* PARASITES IN THE MOUSE LIVER: ROLE OF KUPFFER CELLS

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Leishmania donovani, a kinetoplastid parasite, causes visceral leishmaniasis, a fatal disease if left untreated. *L. donovani* has a digenic lifestyle and alternates from the extracellular promastigotes form in the gut of the sandfly vector to the intracellular amastigote form in macrophages of the vertebrate host where it resides in internal organs, such as the liver, spleen, and bone marrow. Kupffer cells (KCs), macrophages of the liver, are targets of *L. donovani* and play a major role in its clearance. However, the role of the liver in immunoprotection is not completely understood. As potential vaccine candidates, our lab developed attenuated *L. donovani* parasites through gene knock-outs: centrin 1 mutant (LdCen-/-) and p27 mutant (Ldp27-/-). The aim of this study is to evaluate the infectivity and immune response to live-attenuated parasites in KCs *in vitro*. Cells were harvested from the livers of BALB/c mice after perfusion to remove circulating red blood cells and leukocytes. The livers were disrupted to form single cell suspensions, and fractionated through density centrifugation to isolate intrahepatic leukocytes (IHLs). The IHLs were cultured, and KCs were isolated from the IHL population by their adherence to plastic, which averaged ~90% of the IHL population. KCs were characterized by flow cytometry using cell surface markers CD45 and F4/80. These cells were used for *in vitro* infection with *L. donovani* wild-type, LdCen1-/-, or Ldp27-/- parasites. The data on infection as indicated by the parasite number and immune response by measuring NO and secreted cytokines will be presented. Further evaluation of the pathogenesis and immune response to *L. donovani* wild-type, LdCen1-/-, or Ldp27-/- parasites in the liver will be studied by *in vivo* infection of mice.

1243

ACTIVATION OF IRE1-MEDIATED SIGNALING PATHWAY IN CACO-2 CELLS INFECTED WITH *TRYPANOSOMA CRUZI* OR TREATED WITH LPS

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Lipopolysaccharide (LPS) induced TLR-4 activation leading to kinase inositol-requiring enzyme 1 (IRE1)-mediated signaling pathway has been shown to upregulate inflammatory response targets while leaving the canonical unfolded protein response (UPR) targets uninduced. Activation of endoplasmic reticulum (ER) stress sensor IRE1a and its downstream target, the transcription factor, X-box binding protein 1(XBP1) acts synergistically with TLR-4 for optimal and sustained production of proinflammatory cytokines in macrophages**.** UPR is a homeostatic signaling response that attempts to minimize ER-mediated protein folding stress by globally diminishing translation, and increasing the folding and secretory capacity of ER. UPR is typically occurs in metazoan eukaryotes, but a distinct form is observed in kinetoplastids. Conventional UPR proteins, including IRE1a, have not been detected in kinetoplastids by standard ortholog detection tools. We sought to explore the role of *Trypanosome cruzi* and/or LPS in activation of IRE1b, one of the UPR proteins, that is typically expressed in colonic epithelial cells. We prepared extracts of untreated Caco-2 cells, and Caco-2 cells infected with *T. cruzi* (Brazil strain; 1 x 10^7/mL), treated with LPS (100 ng/ml) or cells first infected with *T. cruzi* and then treated with LPS. In each instance, *T. cruzi* was allowed to develop for five days and LPS treatment lasted 12 hours. The cell extracts were subjected to polyacrylamide gel electrophoresis and transferred to polyvinylidane difluoride membrane. The blots were treated with anti-IRE1b which was detected by a chemiluminescence assay. We detected IRE1b protein in extracts of Caco-2 cells after each or both treatments. Our findings suggest that both *T. cruzi* and LPS independently induce IRE1b mediated-ER stress response in Caco-2 cells. This may be a novel innate immune signaling pathway, independent of the classical UPR response, that warrants further investigation.

1244

GENETICALLY MODIFIED LIVE ATTENUATED *LEISHMANIA DONOVANI* PARASITES INDUCE PROTECTIVE IMMUNE RESPONSE *IN VITRO* IN BONE MARROW DERIVED MACROPHAGES FROM YOUNG AND AGED MICE

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Leishmaniasis causes significant morbidity and mortality worldwide and age of the infected individuals appears to be critical in determining the clinical outcome of the infection. Previously we showed that genetically modified live attenuated *Leishmania donovani* parasite cell lines (LdCen$^{-/-}$ and Ldp27$^{-/-}$) induce a strong protective cellular immune response against wild type *Leishmania* infection in mice. In this study we explored whether there is age related difference in immune response to the two live attenuated parasites in bone marrow derived macrophages (BMDM) from young (2 months) and old (18 months) mice. We observed that both these cells lines induce Nitric Oxide (NO) and concomitant suppression of arginase activity, in BMDMs derived from both age groups compared to wild type parasite. The enhanced NO production correlated with the increased phosphorylation of p38MAPK and down regulation of phosphorylation of ERK, components involved in regulating pro-inflammatory cytokine response. Next we evaluated the effect of live attenuated parasite infection on the membrane architecture of the BMDMs from young and aged mice. There is neither quenching of cholesterol from the membranes nor change in membrane fluidity as is observed with wild type infection of such macrophages. In addition there is augmentation of the antigen presentation capability of young and aged mice derived BMDMs by prominently restoring the MHC-II architecture. Overall the study suggests that live attenuated parasites generate an immune response in BMDM isolated from young and aged groups of mice similar to that has been observed in protection against *Leishmania* infections. Therefore these studies suggest that live attenuated parasites can be efficacious irrespective of the age. Future studies will focus on evaluating the efficacy of these attenuated parasites *in vivo* in both young and aged mice against *Leishmania* infection.

1245

IDENTIFICATION OF NEW ANTIGENS FROM *TRYPANOSOMA CRUZI* GENOME BY IMMUNOINFORMATICS ANALYSIS AND THE EVALUATION OF THE RECALL RESPONSE OF SPLENOCYTES FROM INFECTED MICE

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Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*, and activation of CD8+ T cell is crucial for a protective immune response. Therefore, the identification of antigens with epitopes restricted to MHC class I is necessary for vaccine development against *Trypanosoma cruzi*. In

this study, immunoinformatic programs were used to predict epitopes for H-2Dd and H-2Kd. In a first step of the analysis, we identified 172 epitopes with MHC binding potential using the programs NetMHC and RANKPEP. The proteins sequence containing these epitopes were re-analyzed with other programs (SYFPEITHI BIMAS-HLA-I ProPred, MAPPP, ANNPred, compred, SVMHC, IEBD and Preded) to generate a combined consensus of prediction. We selected 26 epitopes that were predicted by the highest number of programs and the best consensus of prediction for validation. We measured the IFNγ recall response of splenocytes from *T. cruzi* infected mice by ELISA following stimulation with the selected peptides. The results show that 10/26 epitopes induce IFNγ production, indicating that these epitopes can cause T cell activation. This suggests that these epitopes are processed and presented by MHC class I by antigen-presenting cells during natural infection by *Trypanosoma cruzi*. 4/10 epitopes confirmed to correspond to proteins with enzymatic functions, and 6/10 correspond to proteins with unknown functions. These epitopes are new potential vaccine candidates against Chagas disease.

1246

DOES CLUSTERING EXIST IN THE TCI DTU OF *TRYPANOSOMA CRUZI,* A REVISION OF THE MINI-EXON GENE (SL-IR)

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Trypanosoma cruzi includes six discrete typing units (DTUs) TcI-TcVI and a seventh one named Tcbat. Previous studies based on intergenic sequences of the mini-exon gene (SL-IR) have identified within TcI, five genotype groups presenting specific epidemiological properties. Given the epidemiological importance of TcI, a DTU that has a very wide geographical distribution throughout the endemic area for Chagas disease, we conducted an exhaustive revision of the sequence variability of the SL-IR, using 244 TcI partial sequences from isolates, cellular or molecular clones, from 11 Latin American countries. First, the evolutionary branching between strains was examined by analyzing only the single nucleotide polymorphism (SNP) deleting the microsatellite region and the gaps. After haplotype reconstruction using the PHASE algorithm, because of the presence of several ambiguous nucleotides in the SNP region, a total of 131 different haplotypes were obtained for network construction using median-joining (MJ) method. The topology reveals how difficult it is to identify an obvious structure in TcI for most of the parameters examined; somewhat genetic and geographical structures exist, but no structure was depicted with cycle and host origins. Then the variability of the microsatellite region (MS), previously used to identify the five TcI genotype groups, was reanalyzed using principal component analysis (PCA); similarly, the two-dimensional projection of the 26 depicted genotypes, does not make it possible to infer clear clustering. Indeed, the long-lasting evolution with possible recombination events, the occurrence of several waves of geographical dispersions (old and recent), and the high flow of strains between sylvatic and domestic cycles partially hide the major evolutionary trends within TcI. Moreover, we identified several problems in previous analyses, and concluded that in absence of supplementary studies of TcI phylogeny with other genetic markers, it is hazardous to use only the mini-exon intergenic region as a relevant marker of the substructure within TcI.

1247

LEISHMANIA DONOVANI UFM1-/- UFSP-/- DOUBLE NULL MUTANTS AS POTENTIAL LIVE ATTENUATED VACCINES

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Leishmaniasis is a spectrum of diseases caused by protozoan parasites belonging to several different *Leishmania* species. There are no effective vaccines against leishmaniases. Recent estimates have shown that an anti-leishmanial vaccine with even a 50% efficacy and protection spanning a 5 year period would still be cost-effective compared to currently available chemotherapies. Previous vaccination approaches including killed *Leishmania* parasites, subunit vaccines or DNA vaccination have not yielded long lasting immunity. We have evaluated genetically attenuated *L. donovani* parasites as vaccine candidates. Such genetically attenuated parasites have been shown to confer robust protection against subsequent *Leishmania* infection in animal models. However, the live-attenuated vaccine approach faces formidable obstacles of demonstration of safety in clinical use. To address the safety of live attenuated parasites, we have developed a *L. donovani* cell line lacking two genes Ufm1 and Ufsp involved in *Leishmania* ubiquitination pathway. Ufm1 (ubiquitin-fold modifier 1) is a ubiquitin like protein that conjugates to parasite proteins in *L. donovani*. This conjugation is initiated by the Ufm1 processing activity of the enzyme Ufsp. Gene deletion experiments have shown that lack of either Ufm1 or Ufsp affect the growth of amastigote stages. The double gene knockout mutants similarly have shown severe growth reduction in the amastigote stage. Infection experiments in Balb/C mice showed a 6-log fold difference in the parasite burden (LdUfm1-/-Ufsp-/- versus wild type) at 8 week post infection in the spleens. These results demonstrate that deletion of Ufm1 and Ufsp genes leads to a strong attenuation of virulence. Further, the use of double gene knockout mutants have the advantage of limiting the probability of recombination and thus reversion to virulent phenotype since the two genes are located on separate chromosomal *loci* (Ufsp on 34 and Ufm1 on 16). Results on the characterization of immunopathology of the double knockout mutants will be discussed.

1248

MOLECULAR EPIDEMIOLOGY OF CHAGAS DISEASE IN SOUTHEASTERN LOUISIANA: ANALYSIS OF SAMPLES FROM PERIDOMESTIC RODENTS AND KISSING BUGS

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The protozoan parasite *Trypanosoma cruzi*, causative agent of Chagas disease, infects at least 8 million people in Latin America and is estimated to cause ~13,000 deaths per year. *T. cruzi* is endemic throughout Latin America and in the southern half of the United States, but Chagas disease occurs primarily in areas where human populations have contact with domestic and peridomestic triatomine vector species associated with infected vertebrate reservoirs. Some autochthonous cases have also been reported in the southern United States. Although bugs are directly involved in the process of active transmission of the pathogen, it is important to consider all the risk factors associated with the vector-host-parasite relationship. Tissues from thirty rodents captured in a rural area of New Orleans, LA were analyzed. The samples, including heart, liver, spleen and muscle, were examined for the presence of *T. cruzi*. We also examined five recently collected triatomines (*Triatoma sanguisuga*). Analysis of > 500 additional triatomine specimens is ongoing and will be reported at the meeting. In the rodents, 100 samples corresponding to different tissues were taken for DNA extraction using QIAmp® DNA extraction kit (Quiagen). The molecular characterization of the tissues obtained was performed by amplifying the kDNA minicircle and the mini-exon gene's intergenic region-SL-IR. Preliminary data showed that 18/30 (60%) rodents were positive for *T. cruzi* and 3/5 (60%) of the triatomines also tested positive. At least three distinct strains of *T. cruzi* were present in the samples. The amplification from *T. cruzi* DNA extracted from tissues showed an interesting mix of infections among TcI and non-TcI, possibly exhibiting a tissue-specific strain association. These results show the importance of determining *T. cruzi* strain associations with different triatomine and vertebrate host species so as to better understand the eco-epidemiology of *T. cruzi* in Latin America and in the southern Unites States. Ultimately, this will bring about improved projections of risk of infection for humans in differing environments.

1249

CONSIDERABLE PROPORTION OF *LEISHMANIA BRAZILIENSIS* RRNA MOLECULES ARE POLYADENYLATED

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Leishmania parasites are ancestral eukaryotes with unusual characteristics like polycistronic transcription and RNA trans-splicing. Like other eukaryotes, their RNA ribosomal genes are tandemly repeated and transcribed by RNA polymerase I. Unlike other eukaryotes, *Leishmania* ribosomes have rRNA molecules of 18S, 5.8S, and 28S, with the latter one being split into six rRNAs (α.γ, β, δ, ζ and ε). The polyadenylation is a post-transcriptional process well known for mRNA but scarcely reported for rRNA. Our previous work on *L. braziliensis* and *L. donovani* demonstrated that at least the rRNA 28S ε undergo the polyadenylation process and that its relative abundance varies in *Leishmania* promastigote and amastigote stages. To determine if all rRNA gene subunits are subjected to polyadenylation, we evaluated the 18S rRNA, 5.8S rRNA and all the subunits homolog to 28S rRNA at stationary and logarithmic phase promastigotes of the *L. braziliensis* strain MHOM/BR/75/M2904. We found that all the rRNA subunits were polyadenylated. Moreover, we quantified the absolute amount of polyadenylated and non-polyadenylated rRNA of the sub-units 18S, 5.8S and 28S α by Reverse Transcription-Real time quantitative PCR. In the logarithmic promastigotes, the percentage of polyadenylated rRNA 18S, rRNA 5.8S and rRNA 28S α were 0.378 ± 0.02 (mean ± standard deviation), 4.55 ± 0.43 and 13.86 ± 0.95, respectively. The stationary promastigotes had higher percentages of polyadenylated rRNA 18S (0.704 ±0.29, *P*=0.064) and rRNA 5.8S (5.69 ± 0.28, *P*=0.045) than the logarithmic promastigotes, whereas the 28S α did not show any significant differences between log and stationary promastigotes. These findings confirm a remarkable fact of *Leishmania* rRNA gene expression (also present in *L.amazonensis*, data not shown) and it is related to the parasite growth. The biological role of this phenomenon remains unknown but its wide conservation in the genus *Leishmania* indicates it is an important one.

1250

PHENOTYPIC CHARACTERISTICS OF ACUTE CHAGASIC MYOCARDITIS AMONG C57 AND BALB/C MICE

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Chagasic disease is a notable neglected tropical disease with high morbidity in Latin America and among immigrants to the US. The primary mechanism of mortality is cardiomyopathy and sudden death. Acute chagasic myocarditis is consistently found in acute infections but little is known about its contribution to chronic forms of cardiomyopathy and what host factors play a role in acute myocarditis. The aim of this study was to phenotypically characterize two strains of mice with differential susceptibility to acute chagasic infection and correlate strain phenotypes with heart tissue gene expression. Laboratory mouse Tulahuen strain of *Trypanosoma cruzi* was grown in 3T3 fibroblast cell culture and tissue-derived trypomastigotes (TCT) were harvested from supernatant. C57 and Balb/c mice were injected intraperiotneally with 0 or 150-200 TCT. Weekly, mice were weighed and *parasitemia* was monitored via retro-orbital blood sample. At 4 weeks Brain natriuretic peptide (BNP) and Troponin were measured in plasma and echocardiograms were obtained. 4-week mortality was 56.3% and 12.5% for Balb/c and C57 (p=0.009), respectively. Infected Balb/c mice lost more weight than infected C57 mice (p=0.018). Parasitemia peaked at 2 weeks, but was not significantly different between strains due to high variation in counts: 500,781 ± 866,464 (Balb/c) vs. 140,625 ± 280,606 (C57) parasites/ml (p=0.12). For infected mice, BNP and troponin levels were not significantly different between strains, but BNP differed from uninfected mice. Echocardiograms demonstrated differences in heart rate in BALB/c vs. C57 mice: 413 vs. 476 bpm, (p=0.0001) and stroke volume: 31.9 ± 9.3 vs. 39.2 ± 5.5 µl (p=0.03); therefore in cardiac output: 13.1 ± 3.5 vs. 18.7 ± 3.2 µl/min (p=0.002). There are relevant susceptibility and hemodynamic differences between these strains of mice during acute chagasic infection. Further characterizations of heart tissue histopathology, immunohistochemistry and gene expression will investigate possible host factor determinants for acute chagasic myocarditis.

1250A

QUANTITATIVE KDNA ASSESSMENT DURING TREATMENT OF MUCOSAL LEISHMANIASIS AS A POTENTIAL BIOMARKER OF OUTCOME

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Mucosal leishmaniasis (ML) is a disfiguring manifestation of infection with *Leishmania* (Viannia) *spp.* As there is no known biomarker of treatment outcome in ML, we evaluated the concentration of kinetoplast minicircle DNA (kDNA) by cytology brush quantitative PCR before, during, and after treatment of ML in Peruvian patients. ML lesions were sampled by cytology brushes for quantitative PCR at enrolment, days 14 and 21_28 of therapy, and 3-, 6-, or 12-mos after treatment. Parasite concentration in tissue was correlated to demographic, clinical, and parasitologic factors. Twenty patients completed follow-up: 12 men and 8 women, with median age of 37 yrs (range 18_78 yrs). Fifteen patients were treated with sodium stibogluconate, and 5 with amphotericin B. Cure was achieved in 17 patients, while 2 patients failed multiple courses of therapy. Clinical outcome is unknown in 1 patient. Mean parasite load (PL) at enrolment was 85,614.8 ± 60,427.3 parasites per ug of tissue DNA (par/ug tDNA). Three patterns of quantifiable kDNA during therapy and follow-up emerged: pattern 1 (N=10) was characterized by a mean PL of 170,867 ± 117,482.6 at enrolment, with sequential decline in PL during and after therapy until kDNA was undetectable. Pattern 2 (N=4) was characterized by mean PL of 566.4 ± 306.4 at enrolment, with clearance of detectable kDNA by D14 of treatment, followed by an increased PL by D21-28 of treatment to 80.4 ± 32.1 par/ug tDNA. Pattern 3 (N=6) was characterized by mean PL of 226.7 ± 116.1 at enrolment, with clearance of detectable kDNA during treatment, followed by increased PL by 6-mos follow-up to 36.6 ± 13.1 par/ug tDNA. Both patients who failed treatment demonstrated Pattern 1. Patterns 2 and 3 were associated with granulomatous inflammation (p=0.02). Younger age (33.5 vs. 64 yrs, p=0.10) and shorter ML duration (20.5 vs. 48 mos, p=0.11) are potentially correlated to sequential clearance (pattern 1). Baseline PL, sex, exposure duration, lesion number, and ML location were not correlated to pattern of PL. We have demonstrated that the concentration of parasite kDNA in ML can be quantified by cytology brush sampling and quantitative PCR during and after treatment. Interim analysis demonstrates 3 distinct patterns of PL during and after treatment, which warrant further investigation. Granulomatous inflammation may predict rebound of PL during or after treatment, though the clinical significance of this rebound is presently unknown.

1251

COMPARISON OF TWO COMBINATION PARASITE LACTATE DEHYDROGENASE-BASED RAPID TESTS FOR THE DIAGNOSIS OF MALARIA DUE TO *PLASMODIUM KNOWLESI* AND OTHER *PLASMODIUM* SPECIES IN SABAH, MALAYSIA

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Plasmodium knowlesi human infection has been reported throughout South-East Asia, and is the most common cause of severe malaria in parts

of Borneo. Microscopic misdiagnosis is common, and may impact prompt initiation of treatment shown to improve mortality outcomes. Previous studies have shown cross-reactivity of *P. knowlesi* with parasite lactate dehydrogenase monoclonal antibodies used to detect *P. falciparum* and *P. vivax*. Our initial evaluation of rapid diagnostic tests (RDTs) has not demonstrated sufficient sensitivity for *P. knowlesi*, and no specific antibody for *P. knowlesi* has been developed. At both tertiary and district referral sites in Sabah, Malaysia, we prospectively evaluated two combination RDTs for the diagnosis of uncomplicated and severe malaria. Firstly with a pan-*Plasmodium* parasite lactate dehydrogenase (pan-pLDH) and *P. falciparum* specific parasite lactate dehyrogenase (PfLDH) RDT (Optimal-IT). Secondly with a non-*P. falciparum* pan-parasite lactate dehydrogenase (VOM), and *P. falciparum* histidine-rich protein-2 (HRP2) RDT (Carestart). Among 250 patients hospitalised with PCR-confirmed *P. knowlesi*, *P. falciparum* and *P. vivax* monoinfection, the pre-treatment sensitivity of the pan-pLDH test for each species was 36% (49/137; 95% confidence interval [CI] 28 to 44%), 75% (63/84; CI 64 to 84), and 83% (24/29; CI 64 to 94) respectively. The PfLDH test sensitivities were 33% (45/137; CI 25 to 41), 77% (65/84; CI 67 to 86) and 14% (4/29; CI 4 to 32) respectively. The VOM component was the most sensitive test for both uncomplicated (44%; 60/137; CI 35 to 53) and severe (79%; 15/19; CI 54 to 94) *P. knowlesi* malaria but remained clinically insufficient. More sensitive RDTs or alternative molecular diagnostic tools are needed in areas of *P. knowlesi* endemicity.

1252

COMPARATIVE ANALYSIS OF MALARIA INFECTIONS BY NESTED PCR USING A POOLING STRATEGY ON DRIED BLOOD SPOTS AND PLACENTAL HISTOLOGY IN MICROSCOPY-NEGATIVE MALAWIAN WOMEN ON IPTP

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Malaria infection in pregnant women on intermittent preventive treatment in pregnancy (IPTp) often presents low parasite densities at delivery and this poses a great diagnostic challenge. In this study, a nested polymerase chain reaction (nPCR) assay for the 18S rRNA gene of *Plasmodium falciparum* was conducted to detect malaria infection in microscopy-negative pregnant women at delivery from an IPTp effectiveness study conducted in Malawi. A sample pooling strategy was developed for screening malaria infection using dried blood spots samples (DBSs) collected from placenta or periphery at delivery. Considering a known malaria prevalence of 7.6% by microscopy in pregnant women at delivery, histologic results were used to stratify the 619 available microscopy-negative samples into sample pools. Each sample pool contained 4 DBSs from histology-positive samples or 10 DBSs from histology-negative samples prior to DNA extraction for first round of nPCR screening. For those nPCR-positive pools, DBSs were then individually extracted and a second round of nPCR assay was performed. Overall, of 619 microscopy-negative DBSs, 179 (28.9%) were positive by histology and 52 (8.4%) were positive by nPCR. Among the histology-positive samples, 39 (21.8%) had active infection (acute and chronic) and 140 (78.2%) had past infection. Using the histology results as a reference, 71.8% women were nPCR-positive in the active infection group, 7.1% were nPCR-positive in the past infection group, and 3.2% were nPCR-positive in histology-negative group. In conclusion, histology diagnosis detected more malaria infection, but nPCR combined with a proper sample pooling strategy is still a practical and sensitive method to detect low density, active malaria infection at delivery. This study has demonstrated that nPCR can be a useful tool to detect submicroscopic malaria infection in pregnant women at delivery when histology diagnosis is not available.

1253

NATIONALLY REPRESENTATIVE SURVEYS OF MALARIA DIAGNOSTIC CAPACITY IN THE PUBLIC SECTOR: FINDINGS FROM GHANA AND BENIN

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In many African settings, malaria cases are treated presumptively. The absence of parasitological confirmation of malaria infection can lead to overtreatment of febrile illness with anti-malarial drugs, or the missing of other potentially fatal conditions. The development of rapid diagnostic tests for malaria (RDTs) combined with the scale up of Artemisinin Combination Therapies (ACTs) has led to increasing pressure to scale up parasitological diagnosis. In order to assess the availability, quality and accuracy of malaria diagnosis in Ghana and Benin, nationally representative health facility surveys were conducted in publicly supported health facilities in both countries. Results indicate that diagnostics are performed accurately a majority of the time when they are applied. The sensitivity and specificity of microscopy compared to expert readings was approximately 80% across all sampled facilities on the day of the survey. Furthermore, all observed RDTs in Ghana were interpreted correctly based on a surveyor's re-interpretation. These results appear significantly better than historic literature on malaria diagnosis with microscopy in many African locations. In Ghana, in the majority of cases, clinicians gave or prescribed drugs in line with test results. While this result is promising, it only reflects practice among patients where a test result was received. Many patients were diagnosed with malaria clinically (i.e. in the absence of any test results). While few of the patients with negative test results received a malaria diagnosis, only half of all fever patients were referred for a malaria test. Despite the overall appearance of the acceptance of testing and the agreement of clinical prescribing practice with laboratory results, approximately 30% of patients who received negative test results still received anti-malarial drugs.

1254

MAGNETIC DETECTION OF HEMOZOIN SURPASSES GOLD STANDARDS OF MALARIA DIAGNOSIS AND RIVALS SENSITIVITY OF MOLECULAR BASED METHODS

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Malaria parasites digest hemoglobin and in the process release cationic alpha-hematin, which is toxic to the red blood cell (RBC) and developing parasite. The developing parasite polymerizes this substance into chemically inert crystals known as hemozoin. Here we have exploited the paramagnetic properties of hemozoin to develop magneto-optical diagnosis (MOD) of malaria. When mixed with water, parasitized RBCs swell, burst open and release hemozoin into solution. Exposure of this lysate to an alternating magnetic field periodically aligns the hemozoin crystals so they block the transmission of light through the solution in proportion to *parasitemia*. When testing MOD on 291 samples from a malaria-endemic area we detected as few as 39 parasitized cells/μL from patients in less than1 minute with an overall accuracy of 93% compared to PCR based detection methods. Additionally, a subset of these patient samples were also compared to RDT (CareStart HRP2/pLDH (Pf/PAN)

COMBO) based detection methods which showed only 29% accuracy when compared to PCR results. Further studies of cultured parasites showed even lower detection of <1 parasitized cells/μL This device provides a rapid, robust, and inexpensive diagnosis of malaria which is an improvement over microscopic and RDT based diagnosis and allows for screenings on a population-based scale which is in line with the goals of global malaria elimination.

1255

SCALING-UP MALARIA RAPID DIAGNOSTIC TESTS AND ARTEMISININ-BASED COMBINATION THERAPY INTO INTEGRATED COMMUNITY CASE MANAGEMENT SITES: RESULTS FROM TWO REMOTE AND LOW-RESOURCE SETTINGS IN THE DEMOCRATIC REPUBLIC OF CONGO

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Integrated Case Management of Childhood Illness (iCCM) improves access to prompt, accurate diagnosis and effective treatment of malaria for populations with limited access to health facilities. In the Democratic Republic of Congo (DRC), a pilot study in 2008-2009 demonstrated the feasibility and the acceptability of integrated use of rapid diagnostic tests (RDTs) for malaria and artemisinin-based combination therapy (ACT) in remote villages by community health workers (CHWs). Scaling-up of the newly adopted strategy began in 2012, reaching currently 129 iCCM sites. This abstract reports the results of the scaling-up in two targeted sites in order to improve their implementation. Patients' forms filed by CHWs from two targeted iCCM sites in Kanda-Kanda health zone were reviewed to assess their adherence to the new malaria treatment guidelines. From July 2012 through March 2013, 644 sick children under five years were managed by CHWs, out of which 432 (67%) were complaining of fever for less than two days without signs of danger. RDTs were performed on 181 (42% of those with fever) children. The remaining uncomplicated cases were treated presumptively with ACTs. CHWs referred 12 severe cases to health facilities for proper case management. Among those tested, 169 (93%) had a positive RDT of which 166 (98%) were treated with ACTs. However, 11 of the 12 patients with negative RDTs were treated also with ACTs. Among those confirmed uncomplicated RDT-positive cases treated with ACTs, 68% were treated within 48 hours of the onset of fever. iCCM has the potential to improve access to prompt, effective management of uncomplicated malaria in remote, low resource settings but challenges remain to improve CHW use of RDTs for diagnosis and adherence to test results.

1256

USING OUTCOME-DRIVEN INNOVATION THEORY TO CLARIFY TARGET PRODUCT PROFILES FOR NEXT-GENERATION MALARIA DIAGNOSTICS

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Diagnostic tools used to reduce the burden of malaria in the control phase are less effective in regions undergoing programmatic reorientation toward malaria elimination. Next generation diagnostics for malaria elimination will need to be more accurate than microscopy and existing rapid diagnostic tests to detect the reservoirs of low-density, asymptomatic infections that perpetuate disease transmission. In addition, new diagnostics will need to be user friendly, field deployable, and capable of high throughput at low cost. Despite ongoing progress in several diagnostic development programs, the technical and market requirements for elimination phase diagnostics remain ambiguous, and therefore developers lack the incentive necessary to bring new technologies to market. To address the need for detailed target product profiles (TPPs) for elimination-specific diagnostics, PATH's project DIAMETER (diagnostics for malaria elimination toward eradication) team has identified a comprehensive list of outcome-based use-scenarios that are critical to the elimination context. Through a review of the literature and stakeholder interviews, we capture the essential system components, performance criteria, and market requirements that define success for malaria elimination stakeholders including health workers, global and national policymakers, and public and private health providers. Our findings will inform recommendations and TPPs to provide clear guidance ensuring the most efficient new diagnostic innovations are accelerated to market to support elimination campaigns.

1257

HIGHLY SENSITIVE RNA-BASED PARALLEL DETECTION OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* ASEXUAL STAGES AND GAMETOCYTES

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For studies requiring highly sensitive and simultaneous quantification of sexual and asexual stages of *Plasmodium falciparum* and *P. vivax*, 18S rRNA transcript-based detection saves efforts or costs. RNA-based positivity is considerably higher than other methods. For simultaneous highly sensitive quantification of both blood stages and gametocytes in an area of equally high prevalence of both *Plasmodium* species, we have compared and optimized different strategies for field and laboratory procedures in a cross sectional survey in 315 5-9 yr old children from Papua New Guinea. qRT-PCR was performed for gametocyte markers pfs25 and pvs25, *Plasmodium* species prevalence was determined by targeting both, 18S rRNA genes and transcripts. RNA-based parasite detection resulted in a *P. falciparum* positivity of 24%; of these 41% carried gametocytes. *P. vivax* positivity was 34%, with 36.4% of these carrying gametocytes. Sensitivity of DNA-based parasite detection was substantially lower with 14.1% for *P. falciparum* and 19.6% for *P. vivax*. Using the lower DNA-based prevalence of asexual stages as a denominator increased the percentage of gametocyte-positive infections to 59.1% for *P. falciparum* and 53.1% for *P. vivax*. Because of its easy measurability in host blood the prevalence of gametocyte carriage can be used to assess the effects of malaria interventions on transmission intensity. With optimized field procedures RNA-based assays were feasible in remote settings. This approach provides in parallel a highly sensitive measure for asexual stage prevalence.

1258

TOWARDS THE DEVELOPMENT OF SALIVA-BASED MALARIA DIAGNOSTICS: MASS SPECTROMETRY BASED IDENTIFICATION OF GAMETOCYTE PROTEINS IN HUMAN SALIVA

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Robust and highly sensitive saliva-based malaria diagnostics, especially for asymptomatic carriage of *Plasmodium falciparum* gametocytes (the only mosquito-transmissible stage), are an important research priority towards the eradication of malaria. Here, we detail our plan to develop such

diagnostics and present data from our baseline proteomic analyses and initial field trials. We first built a comprehensive Mass Spectrometry (MS) workflow to establish updated proteome databases for (a) the human red blood cell, (b) human saliva and (c) *P. falciparum* gametocytes. Using this new baseline information, we developed an optimized MS protocol for determining the limit of sensitivity of gametocyte protein identification in human saliva by spiking extracted proteins from 20-200 gametocytes into 1 uL of healthy human saliva. However, the high abundance of salivary amylases, proline-rich proteins and statherin interfered with MS data-dependent scan mode and the identification of low abundance proteins was difficult. To overcome this limitation, we used peptide ligand library technology (PLLT) to "balance" protein concentrations, which can improve the gametocyte protein detection limit in spiked human saliva by 5 to 10 folds. In collaboration with clinicians in Cameroon, we then used our optimized protocol to analyze both blinded and unblinded saliva to identify candidate biomarkers for asexual and gametocyte stages of *Plasmodium*. Multi-Reaction Monitoring (MRM) will be used in ongoing experiments to validate these candidate biomarkers in individual human saliva samples. We anticipate that the confident, reproducible identification of gametocyte-specific proteins from saliva will compel the production of high-affinity rabbit polyclonal antibodies against the targeted proteins. These antibodies, once validated further by Western blot and Immunofluorescence assays, can then form the basis for the development of prototype gametocyte-specific saliva rapid diagnostic tests.

1259

USE OF MALARIA RAPID DIAGNOSTIC TEST RESULTS AMONG COMMUNITY MEDICINE DISTRIBUTORS IN RURAL UGANDAN COMMUNITIES: IMPACT ON APPROPRIATE TREATMENT

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WHO recommends universal access to malaria diagnostics, and malaria rapid diagnostic test (mRDT) is the only feasible test at community level. Evidence regarding adherence to mRDT results by community medicine distributors (CMDs) and feasibility for use in community case management (CCM) remains limited. We assessed adherence to mRDT results by CMDs in rural Uganda, to provide information that could guide mRDT-based CCM to avoid overuse of artemisinin-based combination therapy (ACTs). A cluster-randomised trial was undertaken to examine the impact and cost-effectiveness of mRDT use by CMDs on the proportion of children receiving appropriate ACT treatment (consistent with parasitological status defined by microscopy on a research slide) in two areas with differing malaria transmission. In each setting, communities were randomised to one of two arms: ACT treatment following mRDT testing (intervention arm) was compared with presumptive treatment (control arm). Data on diagnosis and treatment were recorded by CMDs in treatment registers. Household follow-up interviews and focus group discussions were conducted with CMDs and caretakers of under-five children. Adherence to mRDT results by CMDs exceeded 85% in both transmission settings. In the high transmission area, only 44% of children seen by CMDs in the mRDT arm compared with 99% of patients in the presumptive arm were treated with an ACT, reducing ACT treatment by 55%. Similarly, in the low transmission area, less than 10% of children in the mRDT arm overall were treated with an ACT compared with 94 % in the presumptive arm, reducing ACT prescription by 87%. Analysis of whether this treatment was appropriate treatment (in line with microscopy on a research blood slide) is ongoing, and will be presented. In conclusion, training CMDs in use of mRDT in the context of CMM is feasible. CMDs performed well and adhered to malaria treatment guidelines thus improving rational use of ACTs.

1260

A CLUSTER RANDOMIZED TRIAL INTRODUCING RAPID DIAGNOSTIC TESTS INTO REGISTERED DRUG SHOPS IN UGANDA: IMPACT ON APPROPRIATE TREATMENT OF MALARIA

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WHO recommends universal access to malaria diagnosis, encompassing all treatment providers, including the private sector. Diagnosis reduces inappropriate treatment practices, such as overdiagnosis of malaria and overtreatment with antimalarial drugs. Rapid diagnostic tests (mRDTs) may provide a simple means of confirming malaria diagnosis in drug shops. As yet, there is little evidence of the impact of diagnostic testing on antimalarial drug sales and referral practices by drug shops in Africa. A cluster-randomised trial to evaluate the impact and cost-effectiveness of using mRDTs, compared with presumptive treatment, has been conducted in registered drug shops in Mukono District, Uganda since October 2010. The trial aimed to evaluate the impact of mRDT testing on the proportion of drug shop clients who receive appropriate ACT treatment (consistent with parasitological status defined by microscopy on a research slide). A total of 65 drug shops were randomised to receive training either in use of mRDTs or presumptive diagnosis of malaria. All drug shop vendors (DSVs) were trained on the national malaria treatment guidelines, use of rectal artesunate pre-referral treatment, and when to refer. Supporting interventions included activities to raise community awareness emphasising that not all fevers are malaria and to test blood before receiving or purchasing an ACT. DSVs received close support supervision for first 2 months of implementation. Introduction of mRDTs in drug shops was acceptable to DSVs, the community and health staff. Adherence to mRDT results by DSVs was high with 92% of treatment decisions being consistent with mRDT test results, reducing sales of ACTs by approximately 40%, compared to drug shops in the control arm (presumptive diagnosis). Overall, appropriate treatment in drug shops using mRDTs was significantly higher than in drugs shops using presumptive diagnosis (70.1% versus 33.5%, P=0.0001). In conclusion, introducing mRDTs in drug shops was feasible and acceptable; and had a substantial impact on appropriate treatment of malaria.

1261

BURDEN OF MALARIA IN HIV POSITIVE PERSONS IN A MALARIA ENDEMIC AREA

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In endemic areas, malaria is usually diagnosed presumptively despite the WHO recommendation that malaria diagnosis be parasite based. HIV increases susceptibility to malaria with the result that HIV +ve persons are treated for presumed malaria very frequently. In a cross-sectional study, 2082 people living with HIV (PLWHIV) were evaluated for presence of malaria parasite by expert malaria microscopy of Giemsa stained thick blood film over a one year period. Study population was drawn from the HARVARD partnered President's Emergency Plan for AIDS Relief (PEPFAR) funded APIN adult ARV outpatient clinic, University College Hospital, Ibadan in south-western Nigeria were malaria transmission is intense. The mean age of enrolees was 36.7 ± 9.1 years (range 16-70). 81.5% (1696/2082) were female. The prevalence of malaria *parasitemia* was 15.8% (329/2082). Female PLWHIV was significantly more likely to be parasitemic than her male counterpart (13.9% versus 1.9% p<0.0001). The higher the level of education the less likely it is for patent *parasitemia*. Almost half (49.2%; 1024/2082) of the study population had one symptom or another at enrolment. The 5 most common symptoms were

Fever (70.1%), headache (63.2%), loss of appetite (44.3%), abdominal pains (32.1%), chills and rigors (22.3%) and vomiting (22.3%). Vomiting was the only symptom significantly associated with patent *parasitemia*. Temperature >37.4^{O}C was not significantly associated with malaria *parasitemia*. 43.9% had received antimalarial drugs in the preceding three months. 251 (12.1%0 reported three or more attacks of presumptively diagnosed and treated malaria in the same time frame. Thirty five (35/251; 13.9%) of these claimed to have had 6 to 10 episodes each. Drug use history in the two weeks before enrolment include antibacterial agent (15.6%), antimalarial drugs (34%) with 9% haven taken chloroquine, 12% had ACT and 12.2%sulfadoxine-pyrimethamine. 316 (15.2%) of the PLWHIV believed that they had malaria more frequently and each attack was more severe before their HIV status changed. In conclusion, malaria *parasitemia* is less frequent than earlier believed and parasite - based diagnosis will reduce over treatment with antimalarial drugs.

1262

RAPID DIAGNOSTIC TEST (RDT) PERFORMANCE OF THE MALARIA GOLD MINING PROGRAM IN SURINAME: COMPARING THE PERFORMANCE OF TWO RDT'S

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Good Rapid Diagnostic Test (RDT) performance is at the cornerstone of the malaria diagnoses in Suriname. This because malaria infections occur mainly among persons (ca. 15,000) engaged in small-scale gold mining and related activities. Because this mining areas are remote diagnoses is primarily done by RDT either in the goldmines or in the city, in the gold miners' neighborhood, at the Tourtonne laboratory where testing occurs. End of 2011 the RDT test used switch from Binax to the use of Care tart. To assess the difference in performance of these tests from both test 82 RDT results were compared with microscopical examination by Tourtonne Laboratory (TL). Data was collected from may 2012 till April 2013. The 82 Binax results, compared with microscopy, gave a sensitivity of 76.5% compared to 94.19% for CareStart. The specificity was for both Care Start and Binax 96.9%. A PPV of respectively 86.7% and 88.9% was calculated for Binax and Care Start. Not wanting to miss positive cases the false negative rate found was 6.3% for Binax compared to 1.6% for Care Start with plasmodium *vivax* being the species being missed in both cases. Looking at the performance of the CareStart RDT test in regards to the sensitivity, specificity, PPV and the false negative rate, the Care Start has proven in the Surinamese setting to give a much better performance than the Binax. In this regard the switch from the use of Binax to Care Start was a justified and wise choice.

1263

NEW PULSE ASSAYS THAT MIMIC *IN VIVO* EXPOSURE REVEAL DIFFERENCES IN SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO ARTEMISININ

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Artemisinins (ARTs) are the most effective class of antimalarials against *Plasmodium falciparum*. However, ART resistance has emerged in regions along the Cambodia-Thailand border and many patients in that area experience delayed clearance of blood parasites. Frustratingly, the parasites isolated from those patients do not always show reduced ART sensitivity in standard 3-day *in vitro* assays. Since ART antimalarials have short *in vivo* half-lives of 1 to 2 hours, we have applied short drug pulses to parasites in an effort to better mimic *in vivo* conditions. We hypothesised that short drug pulses may reveal stage and strain-dependent differences in drug sensitivity that are not apparent in standard 3-day assays. We have examined and compared the two laboratory strains D10 and 7G8. In standard 3-day assays, D10 has 2-3 fold higher ART IC_{50} values than 7G8 parasites. In pulse assays, tightly synchronised parasites were subjected to 4 h drug pulses at different stages throughout the intraerythrocytic asexual life cycle. Parasite viability following drug exposure was monitored in the cycle following the drug pulse by flow cytometry using the nucleotide-binding dye, Syto61. Under these conditions, the 7G8 strain exhibited up to 100 fold higher ART sensitivity than D10 parasites. Furthermore, parasites treated with 4 h drug pulses showed significant stage-dependent differences in drug sensitivity. Very early rings (less than 6 h post invasion) were very sensitive to ART. Apart from this very early stage, most of ring stage parasites (mid-ring to late-ring) were relatively insensitive to ART compared to trophozoites and schizonts. We have applied pulse assays to field isolates from the Pailin region in Cambodia that exhibit similar ART IC_{50} values in standard 3-day assays. Our results show that pulse assays can reveal large differences in sensitivity of field strains to ARTs that are not evident in standard assays, which may be clinically relevant.

1264

STATUS OF CHLOROQUINE RESISTANT HAPLOTYPES IN *PLASMODIUM FALCIPARUM* PARASITE POPULATIONS COLLECTED IN POST-EARTHQUAKE HAITI

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Haiti is located on the island of Hispaniola, the last remaining Caribbean island with endemic malaria. In Haiti, chloroquine (CQ) remains the first-line treatment for malaria. Given the challenges of conducting *in vivo* drug efficacy trials in low endemic settings such as Haiti, molecular surveillance for chloroquine resistance markers in the pfcrt gene is useful to identify emergence of resistant alleles in the population. After the January 2010 earthquake, enhanced malaria surveillance was rapidly instituted to monitor for CQ resistance and contain the spread of disease. In this study, 349 bloodspots were collected from suspected malaria cases mostly in areas in and around Port-au-Prince from March through July of 2010. We investigated the CQ resistant pfcrt markers for 121 *Plasmodium falciparum* PCR-positive samples. DNA sequencing of the pfcrt gene covering codons 72-76 was performed on PCR amplified samples. Among a total of 110 samples able to be sequenced, 108 samples were wild-type (CQ sensitive, CVMNK) while only two samples were of a resistant haplotype (CVIET). To determine if these resistant parasite alleles were imported from other endemic countries we conducted a population structure analysis using seven neutral microsatellite markers. This analysis revealed that one of the CQ resistant samples had a neutral multi-locus genotype distinct from all other Haitian samples. We were unable to amplify the other resistant parasite sample for the neutral markers. Cluster analysis of neutral microsatellite data using Structure v2.3 revealed population sub-structure with at least five distinct clusters among the CQ sensitive parasites. Furthermore, genotypes of the CQ sensitive parasites were unique to Haiti when compared to the genotypes of parasites collected in Honduras, Nicaragua, and a number of South American countries. These findings suggest a nonexistent or a very low level of CQ resistant alleles in Haiti, supporting current recommendations to use CQ as first-line treatment, while emphasizing a need for continued molecular monitoring for the emergence of antimalarial resistant parasite populations.

1265

GENETICALLY DISSECTING THE *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER: EVALUATING FUNCTION AND EVOLUTION OF ANTIMALARIAL DRUG RESISTANCE

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As few as four amino acid changes in the *Plasmodium falciparum* Chloroquine Resistance Transporter (PfCRT) are required to mediate malaria parasite resistance to chloroquine (CQ). An example is the Ecu1110 parasite (Ecuador) whose *pfcrt* allele is comprised of K76T, A220S, N326D, and I356L, the necessary determinant being K76T. However, in its evolution, *pfcrt* has accrued additional mutations likely to balance the requirements of enhanced drug resistance and overall parasite fitness. The current understanding of these mutations only depicts the binary states of CQ resistance and CQ sensitivity, however the contribution of each mutation in parasite fitness and drug susceptibility is unknown. Additionally, the order in which these mutations appeared is likely nonrandom, given the rarity of the emergence of CQ resistance. Using Zinc-Finger Nuclease (ZFN) technology as a tool for reverse genetics, we have edited *pfcrt* to recreate the possible evolutionary trajectory of the Ecu1110 *pfcrt* in its transition from CQ sensitive to CQ resistant. We have also investigated additional mutations to recreate the evolutionary path that parasites from Papua New Guinea might have taken as CQ was introduced in the 1950s, leveraging recent genotyping studies of old archived samples. In regenerating historical *pfcrt loci*, our goal is to narrow the possibilities of evolutionary trajectories *pfcrt* has taken to achieve CQ resistance in order to get a clearer understanding of the contributions of not only each mutation but also of each domain of PfCRT in both fitness and function. To further this, we are also using this technology to introduce novel mutations in the vacuolar loop of PfCRT, suspected to be involved in redox sensing, in order to interrogate its native function. Our studies provide further insight into the evolution of *pfcrt*-mediated CQ resistance in the context of fitness constraints, and suggest a role for this protein in maintaining solute homeostasis in the digestive vacuole.

1266

FIELD VALIDATION OF CANDIDATE MOLECULAR MARKERS OF ARTEMISININ RESISTANCE IN MYANMAR

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The emergence and spread of artemisinin-resistant *Plasmodium falciparum* in Southeast Asia threatens malaria control efforts worldwide. Molecular markers of artemisinin resistance, which can be easily assayed at minimal cost, will be critical for directing surveillance and containment. A recent genome-wide association study using samples from Bangladesh, Thailand, and Cambodia identified two SNPs (MAL13-1718319 "MAL13" and MAL10-688956 "MAL10") strongly associated with delayed parasite clearance after treatment with artesunate. To validate the use of these target SNPs as potential markers of resistance, we analyzed dried blood spots obtained during a 2010 WHO Dihydroartemisin-piperaquine Therapeutic Efficacy Survey in Thanbyuzayat, Myanmar for the presence of artemisinin resistance-associated candidate SNPs. DNA was extracted from 68 dried blood spots collected prior to treatment and genotyped for the SNPs on MAL10 and MAL13 using pyrosequencing. Data were corrected using a standard curve to best estimate true ratios of sensitive to resistant genotypes for each sample. Preliminary results are as follows. For MAL10, of 66 samples successfully extracted, 21 (32%) samples contained parasites with the resistant allele, of which 18 samples (27%) were pure resistant and 3 (5%) contained both sensitive and resistant alleles. 45 samples (68%) had only sensitive MAL10 alleles present. All samples had the sensitive allele at MAL13, suggesting that the resistant allele is absent or present at very low levels in the population. Using *parasitemia* on day 3 as a surrogate for delayed parasite clearance, we analyzed the association of the MAL10 resistant allele with this phenotype. 38% (8/21) samples with resistant MAL10 had *parasitemia* on day 3, while 11% (5/45) of sensitive samples had day 3 *parasitemia*. Logistic regression indicated that the resistant MAL10 genotype was a significant predictor of *parasitemia* on day 3 (Odds ratio 4.92, p=0.015). When adjusting for log-transformed day 0 *parasitemia* in the model, the MAL10 resistant SNP became a marginally significant predictor of day 3 *parasitemia* (Odds ratio 3.90, p=0.08). MAL10 may be a valuable marker of delayed parasite clearance and should be investigated further to validate its predictive capability.

1267

HIGH PREVALENCE OF DHFR AND DHPS RESISTANCE HAPLOTYPES FIVE YEARS AFTER REMOVAL OF SULFADOXINE-PYRIMETHAMINE AS THE FIRST-LINE TREATMENT FOR UNCOMPLICATED MALARIA IN MALAWI

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Less than a decade after the replacement of chloroquine with sulfadoxine-pyrimethamine (SP) as the first-line treatment for uncomplicated *Plasmodium falciparum* malaria in Malawi, chloroquine-sensitive parasites re-expanded in the population, to the point of renewed chloroquine clinical efficacy. Decreasing clinical efficacy due to spreading resistance mutations in dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) caused SP to be replaced by an artemisinin-based combination therapy in 2007. Whether SP resistance in Malawi will decline in the absence of drug pressure remains unknown. Here, we report the maintenance of a high prevalence of SP resistant haplotypes five years after the removal of SP as the first-line treatment of uncomplicated malaria in Malawi. Resistance *loci* at dhfr codons 51, 59, 108, and dhps codons 437, 540, 581 were genotyped from 689 infections from 1999-2001 and 893 infections from 2012. Haplotype prevalence was estimated for both time points. SP-sensitive parasite haplotypes were not found at either time point. The prevalence of dhfr 51I/59R/108N triple mutants and dhfr 51I/108N double mutants did not change significantly between time points (85%-88%, p=0.29 and 4%-7%, p=0.06, respectively), although a decrease in dhfr 59R/108N double mutants did occur (38%-0%, p<0.001). An increase in the prevalence of dhps 437G/540E double mutants (84%-96%, p<0.001) and dhps 437G/540E/581G (0%-4%, p<0.001) was observed. The prevalence of dhps A437/K540 SP-sensitive parasites decreased from 7%-1% (p<0.001). These results suggest that although some SP-resistant haplotypes did decrease in prevalence the removal of SP as the first-line treatment of uncomplicated malaria was not sufficient to effect a return of SP-sensitive parasites. Possible explanations for these

findings include minimal fitness cost of resistant haplotypes in the absence of strong SP-drug pressure, sustained selection by the prophylactic use of SP in pregnancy and trimethoprim-sulfamethoxazole in HIV+ individuals, and/or the fixation of dhfr 108N.

1268

DIHYDROPTEROATE SYNTHASE 581 MUTATION IS ASSOCIATED WITH PARASITEMIA AT DELIVERY IN WOMEN WHO RECEIVED INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE

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Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended for the control of malaria in pregnancy. Parasite resistance due to mutations in *Plasmodium falciparum* dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthase (*Pfdhps*) threatens its effectiveness. The *Pfdhps*581G mutation has been associated with increased placental inflammation among women receiving IPTp-SP. HIV-uninfected women with a singleton pregnancy were enrolled at delivery. Peripheral blood and placental samples were collected. Birth weight and gestational age (assessed by Ballard exam) were recorded. Nested polymerase chain reaction (nPCR) for 18S rRNA gene was done for detection of malaria; positive samples were sequenced to determine genotype at *dhfr* and *dhps loci*. We estimated a density of 20 parasites/µl for PCR positive, smear negative samples. PCR was positive in 91 of 710 samples. Genotype data for *dhps*581 were obtained for 81 samples. Of these, 10 were mutant (14%) and 71 were wild type (WT). With the exception of three samples that were not amplified at *dhfr*108, all mutant samples were mutated at *dhfr* codons 51, 59, 108 and *dhps* codons 437 and 540. All samples with *dhps*581G and 69% WT samples were from women who had two doses of IPTp-SP. The *dhps*581G mutation was associated with a positive smear (maternal peripheral, placental, or cord) at delivery (adjusted prevalence ratio (aPR) 3.0, 95% CI 2.0-4.4), even after adjusting for timing of last SP dose. *Pfdhps*581G was associated with increased parasite densities in both maternal peripheral (267 parasites/µl, 95% CI 67-1055 for mutant vs. 39 parasites/µl, 95%CI 28-54 for WT, p=0.0002) and placental (112 parasites/µl, 95% CI 43-290 for mutant vs. 36 parasites/µl, 95% CI 27-50 for WT, p= 0.01) samples. The presence of *dhps*581G was not associated with an increased risk of maternal anemia (aPR 0.55, 95% CI 0.21-1.44), histologically-confirmed placental malaria (aPR 0.90, 95% CI 0.59-1.38), a composite outcome of LBW, preterm delivery, or small for gestational age (aPR 1.48, 95% CI 0.73-2.98), or a significant change in mean birth weight (p=0.77). The *dhps*581G mutation, when present in addition to the quintuple dhps/dhfr mutant, is associated with increased maternal peripheral and placental parasite densities among SP recipients. Monitoring the prevalence of dhps581G is critical in areas where IPTp-SP is used.

1269

SELECTION OF CYTOTOXIC RESISTANCE TO A REVERSED CHLOROQUINE COMPOUND IN *PLASMODIUM FALCIPARUM*

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Antimalarial "reversed chloroquines" are comprised of a chloroquine-like moiety and a resistance reversal-like moiety, and show excellent potency against multi-drug resistant strains of *Plasmodium falciparum*. The dipyridyl analog DM1157 is highly potent against chloroquine-resistant parasites *in vitro* and *ex vivo*. It also showed good oral availability and was curative in 9/10 *P. berghei*-infected mice at doses equimolar to those at which chloroquine is effective. Like chloroquine, DM1157 inhibited beta-hematin formation *in vitro* and hemozoin formation in the parasite. While DM1157 may share a similar mechanism of action to chloroquine, it is not affected by the same *pfcrt* mutations that cause resistance to chloroquine, most significantly the K76T polymorphism. The potential for resistance to reversed chloroquines is unknown, thus we are attempting *in vitro* DM1157 resistance selection in the Dd2 line of *P. falciparum* using 24 hour on-off selection with incrementally increasing concentrations of the compound. Following drug removal, parasites are allowed to recover to 3% *parasitemia* before the next round of selection. After 48 rounds of selection, parasites showed a 2-3 fold increase in the DM1157 IC_{50}, and a 2 fold increase in the chloroquine IC_{50}, and negative cross-resistance with mefloquine. Continued selection has not resulted in further increases in IC_{50}s; rather a 4 fold increase in the LD_{50} emerged after 69 rounds of selection, indicating resistance to cytotoxic effects of the drug. The resistant parasites also showed a slow-growth phenotype. The results suggest that cytotoxic resistance to DM1157 comes at a cost of fitness, seen as slower rates of culture expansion. Parasite cloning and preparation for whole genome analysis is currently underway in order to identify the genetic determinants of resistance and parasite fitness.

1270

EVALUATION OF COARTEM TREATMENT FAILURES IN WEST AFRICA

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Because of concern about potential resistance (prolonged parasite clearance times) in Southeast Asia and the potential for artemisinin resistance, we have examined the effectiveness of Coartem for the treatment of uncomplicated *Plasmodium falciparum* malaria in three communities in West Africa (Gambissara in The Gambia, Dioro in Mali and Thiès in Senegal). These studies have enrolled participants 2-15 years of age with 2,000 to 199,999 asexual parasites per µl of blood who had no evidence of severe or complicated malaria and no medical problems which required treatment other than malaria. Primary endpoints for this study include asexual parasite counts <25% of baseline by day 3, clearance of all asexual parasites by day 7 and the lack of recurrent infection between days 8 and 42. Secondary endpoints include asexual parasite clearance times, ex vivo determinations of susceptibility/resistance to antimalarials such as the artemisinins and their derivatives, amodiaquine, chloroquine, quinine and pyrimethamine; testing for drug resistance markers and for presumptively

neutral markers (barcode assays). These studies have now enrolled 171 subjects with uncomplicated *P. falciparum* malaria, who have been treated with Coartem and followed for recurrent infection or other evidence of treatment failure. Of the 171 subjects enrolled, 8 have been lost to follow-up and 13 have developed recurrent infection between days 8 and 42, although there have been no early treatment failures (on or before day 7). Twelve of 13 subjects with recurrent infections had parasites at the time of recurrence with different genetic markers. The thirteenth subject had parasites with similar markers at the times of diagnosis and recurrence and delayed parasite clearance on day 3. That patient was therefore classified as having antimalarial resistance. Apart from that subject, the results obtained thus far provide no evidence for artemisinin or Coartem resistance at the community level in The Gambia, Mali or Senegal.

1271

SELECTION OF *PLASMODIUM FALCIPARUM* RESISTANCE TO ANTIMALARIAL ACRIDONES

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The need for potent antimalarials to prevent the emergence of drug resistant *Plasmodium falciparum* is urgent. Discovery of novel acridone chemotypes has shown promise for a new antimalarial drug treatment. Dual-function acridones (chemotype II) are N10 substituted, which targets the molecule to the parasite digestive vacuole where they inhibit hemozoin formation and synergize potency of other antimalarials such as quinine and piperaquine. However, the molecular target(s) of broad-spectrum (chemotype I) acridones with efficacy against both liver and blood stage malaria are unknown. Therefore, selection of acridone resistance may lead to identification of a molecular target and the mechanism of action. Using the Dd2 line and the chemotype I compound, T13, we selected stable acridone resistance by using multiple rounds of incremental, 24 hour on-off selection, followed by continuous pressure up to three times the IC_{50} value. Parasites not exposed to the initial on-off pressure have failed to develop resistance while under continuous T13 selection, thus far. A similar strategy with the chemotype II acridone, T16.5, has failed to produce resistance. Control parasites showed an T13 IC_{50} value of ~18 nM, while clonal resistant parasite lines showed an average IC_{50} of 670 nM. Cross-resistance was seen with other chemotype I acridones, but not chemotype II acridones, indicating the importance of the N10 substitution in avoiding the resistance mechanism. Only slight cross-resistance to atovaquone was seen in T13-resistant parasites. This suggests that T13 may target a unique component of the mitochondrial electron transport chain from atovaquone, which specifically inhibits ubiquinone binding to the Q_o site of the cytochrome *bc*1 complex. T13-resistant parasites are being prepared for whole genome sequencing to identify the target molecule(s) of acridone resistance.

1272

ARTEMETHER-LUMEFANTRINE, ARTESUNATE+AMODIAQUINE AND DIHYDROARTEMISININ-PIPERAQUINE FOR TREATING UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN UNDER-FIVE NIGERIAN CHILDREN: A RANDOMIZED CONTROLLED TRIAL

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Plasmodium falciparum is responsible for over 90% of malaria infections in Nigeria, and accounts for 30% of under-five deaths. Uncomplicated malaria in under-five children could rapidly deteriorate to severe fatal malaria if treatment is delayed or ineffective. This paper reports the findings from Afokang, one of two Nigerian sites in a multi-centre African study of the efficacy and safety of three artemisinin-based combination treatment regimens in under-five children. Trial design was open-label, parallel group randomized controlled trial. Children aged 6-59 months with uncomplicated malaria that fulfilled eligibility criteria were randomized to receive arthemether lumefantrine (AL), artesunate + amodiaquine (ASAQ) or dihydroartemisinin-piperaquine (DHAPQ). Participants were actively followed up for 28 days and then passively for 6 months. PCR was performed to distinguish recrudescent parasitaemia from new infections. Intention to treat and per protocol analysis were performed for primary outcomes assessed by D28. A total of 92, 92 and 77 eligible children were randomized to AL, ASAQ and DHAPQ groups respectively. The unadjusted D28 cure rates for AL, ASAQ and DHAPQ were 96.6% (84/87), 94.0% (78/83) and 93.1% (67/72) respectively; with PCR-adjusted D28 cure of 97.7% (84/86) for AL, and 100% for both ASAQ (80/80) and DHAPQ (70/70)..Unadjusted D63 cure rates for AL, ASAQ and DHAPQ were 93.1% (81/87), 92.8% (77/83) and 93.1% (67/72) respectively; with PCR-adjusted D63 cure of 98.8% (83/84) for AL and 100% for both ASAQ (80/80) and DHAPQ (70/70). The PCR-adjusted D28 cure rate of DHAPQ was not statistically significantly different from those of AL (OR: 0.24; 95% CI 0.00,13.25) and ASAQ (OR: 1.14 ; 95% CI 0.01,200.69). As these cure rates exceed 95%, all drugs tested meet the WHO criteria for an effective ACT. Serious adverse events were few (n=4); all four in the ASAQ group but not related to drug effects. These results confirm the appropriateness of continued use of AL and ASAQ in this locality, and have programmatic implications for wider use of DHAPQ in the country.

1273

CHARACTERIZATION OF THE BC1 QI SITE AS A NOVEL ANTIMALARIAL TARGET

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Malaria is a tropical disease that exerts a staggering impact on health and economic productivity, due in part to the emergence of *Plasmodium* drug resistance. To counter the spread of drug resistance, the identification of novel antimalarial targets, especially those that are vital and conserved throughout the *Plasmodium* life cycle, has become a major focus of drug

development. Here, we introduce a subset of endochin-like quinolones (ELQs) that appear to inhibit the reductive (Q_i) site of the mitochondrial cytochrome bc_1 complex. The Q_i site represents a previously unreported antimalarial target that is unaffected by atovaquone resistance mutations at the *bc1* oxidative (Q_o) site, and is compatible with high potency, broad-stage antimalarial activity. Preclinical candidate ELQ-300 was used to generate resistant parasites under incremental drug pressure. Isolated clones were 10-20 fold less sensitive to ELQ-300, and contained a point mutation in the mitochondrially encoded cytochrome *b* gene. This mutation (resulting in Ile to Leu change at position 22) maps close to the cytochrome bc_1 Q_i site and has not been observed in any other malaria parasite resistant to cytochrome bc_1 complex inhibitors. Screens against the ELQ-300 resistant "D1" clone were used to identify additional potentially Q_i-selective ELQs and to pinpoint chemical features that contribute to Q_i targeting. The strongest evidence for Q_i site activity was found for ELQs with bulky chemical groups at the 6-position. Many of these sterically hindered ELQs were 100-1000 fold less potent against the D1 clone. Conversely, D1 cross-resistance was completely absent in ELQs containing small 6-position groups such as fluorine or hydrogen. ELQs containing these smaller groups retained full potency against both ELQ-300 and atovaquone resistant parasites, suggesting that a subset of ELQs are capable of circumventing ELQ-300 resistance at the Q_i site. These results provide compelling evidence that subtle structural features of the *bc1* complex influence targeting and selectivity by quinolones.

1274

IMPACT OF ARTEMISININ BASED COMBINATION THERAPY (ACTS) REPEATED TREATMENT ON THE PREVALENCE OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MOLECULAR MARKERS (*PFCRT* AND *PFMDR1*)

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ACTs are currently used as the malaria first-line treatment in most endemic countries. The aim of this study was to assess the impact of repeated treatment with AS + AQ and AR-L on *Pfcrt* and *Pfmdr1*, in a 3 years randomized clinical trial in Bougoula (Mali). We use WHO 28-day standard in-vivo protocol. Overal 521 blood spoted filter papers were analyzed; mutations frequencies on *Pfcrt* and *Pfmdr1* genes were compared before and after intervention. In the AS + AQ arm we observed a base line frequency of 41.6% against 77.1% for *Pfmdr1-86Y* during the first episode and > 93% in the second, third and fourth episodes of malaria. For the *Pfcrt76T gene* we observe a baseline frequency of 58.9% against 88% during the first episodes and > 93% in the next episodes. For the AR-L arm's, we obtained a baseline frequency of 41.6% against 6.2%, 18.2%, 7.1% and 0% on *Pfmdr186Y gene* for episodes 1, 2, 3 and 5 respectively. Concerning *Pfcrt76T* gene the base line frequency was 58.9% against 59.1%, 75% and 88.8% for episodes 1, 2 and 3 respectively. This study demonstrate that there is a significant increase in *Pfmdr-86Y*, and *Pfcrt-76T* mutants after treatment with AS + AQ and a significant decrease of *Pfmdr1* mutations after treatment with AR-L. Despite the presence of artemisinin,the CTAs select the molecular markers of resistance to the partner molecule.

1275

EVALUATION OF DIAGNOSTIC PLATFORMS FOR G6PD DEFICIENCY INCLUDING TWO QUANTITATIVE TESTS, THE FLUORESCENT SPOT TEST, A POINT-OF-CARE TEST AND A CYTOCHEMICAL STAINING-BASED ASSAY

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A key barrier to achieving elimination of malaria caused by *Plasmodium vivax* infection is effective treatment. There is currently only one class of drugs, 8-aminoquinolines, which can entirely clear the parasite from a patient (radical cure). Unfortunately, patients with a common human trait, glucose-6-phosphate dehydrogenase (G6PD) deficiency, are at high risk of experiencing severe adverse side effects with this class of drugs. Point-of-care G6PD tests are needed to promote safe access to these drugs for patients with malaria. PATH is working with national malaria programs, manufacturers, and other key stakeholders to accelerate development and introduction of point-of-care G6PD tests where they are most needed. As part of this initiative, we evaluated different assays and platforms for determining the G6PD status of a patient. We compared the performance of a lateral flow-based G6PD test, a fluorescent spot test, and two quantitative tests for G6PD deficiency. We provide sensitivity and specificity performance data for these tests and highlight discordant test results. Discordant test results are discussed in the context of sequencing data. Additionally, we show the utility of a cytochemical staining-based assay for determination of G6PD status in addition to identification of females with heterozygous traits for G6PD.

1276

MASS DRUG ADMINISTRATION FOR THE CONTROL AND ELIMINATION OF *PLASMODIUM VIVAX* MALARIA: AN ECOLOGICAL STUDY FROM JIANGSU PROVINCE, CHINA

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Recent progress in malaria control has caused renewed interest in mass drug administration (MDA) as a potential elimination strategy but the evidence base is limited. China has extensive experience with MDA but it is not well documented. We conducted an ecological study to describe the use of MDA for the control and elimination of *Plasmodium vivax* in Jiangsu Province and explore the impact of MDA on malaria incidence. We focused on two periods: 1973–1983 when malaria burden was high and MDA administered to entire counties, and 2000–2009, when malaria burden was low and a targeted approach was used in two counties. We collected all available data about the strategies implemented, MDA coverage, co-interventions, incidence, and adverse events. From 1973–1983, MDA with pyrimethamine and primaquine was used on a large scale, with annual peak coverage reaching almost 30 million people (50% of the population). Joinpoint analyses identified declines in annual incidence, -56.7% (95% CI -75.5 to -23.7%) from 1973–1976 and -12.4% (95% CI -24.7 to 2.0%) from 1976–1983. Population average negative binomial models identified a relationship between higher MDA coverage and lower monthly incidence from 1973–1976, IRR 0.98 (95% CI 0.97 to 1.00), while co-interventions, rainfall, and GDP were not associated. From 2000–2009, MDA using chloroquine and primaquine was targeted to villages and/or individuals residing near passively detected index cases (median 0.04% population coverage) and incidence declined (annual change -43.7 to -14.0%). Safety data were not collected systematically but there were rare reports of

serious but non-fatal events. In Jiangsu Province, China, large scale MDA was associated with declines in high *P. vivax* malaria transmission and a targeted approach likely contributed to interruption of transmission. MDA should be considered a key strategy for malaria control and elimination.

1277

A SAFETY MONITORING TOOL FOR PRIMAQUINE USE TO REDUCE TRANSMISSION OF *PLASMODIUM FALCIPARUM*

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In 2012, the World Health Organization published new guidelines recommending the addition of a lower single dose of primaquine (PQ) (0.25 mg base/kg) than previously recommended as gametocytocidal treatment for *falciparum* malaria in areas threatened by artemisinin resistance and in settings targeting elimination. However, concerns over the small but real risks of drug-related hemolysis associated with the administration of PQ, especially in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals, have restricted its widespread use. In response, this study was designed to support the safe roll-out of low dose PQ through safety monitoring for the treatment of *Plasmodium falciparum* infections to reduce transmission. We developed a tool offering enhanced monitoring to evaluate the safety and tolerability of PQ use in malaria endemic settings. The tool can assist programs to either establish a passive reporting system for adverse events related to low dose PQ therapy or a protocol for enhanced monitoring that actively tracks hematologic response. Confirmed, uncomplicated *falciparum* malaria cases prescribed PQ across all age groups from public and private health facilities are included. Assuming a population prevalence of G6PD deficiency between 5-10%, programs will need to follow 250-500 PQ treated individuals in order to detect a 25% or greater reduction in hemoglobin (Hb) between enrolment and day 7 for G6PD deficient patients with a Type I error of 0.05. For programs engaged in enhanced monitoring, follow-up visits are performed on or near day 7 after enrolment. Data on patient characteristics, malaria diagnosis and treatment, Hb levels and reported adverse events through history taking and physical examination is gathered. Finger-prick blood samples are taken for Hb, to test for G6PD deficiency, and to collect a dried blood spot. All participants are instructed on identifying symptoms of commonly reported adverse events and to monitor the color of their urine. The tool will be piloted in two low transmission countries in the Asia Pacific region and southern Africa. The design of our safety monitoring tool will be presented and could prove useful for programs planning wide-scale roll-out of single low dose PQ use in routine malaria treatment.

1278

A POSSIBLE THREAT TO THE ELIMINATION: OVERVIEW OF IMPORTED MALARIA IN JIANGSU PROVINCE, P.R. CHINA

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The great successful progress has been achieved in P.R. China, since the malaria elimination program launched on 2010. However, there still remain some possible threats, for example, the overseas imported cases significantly increased over the past few years. In terms of a possible resurgence of the disease, a retrospective study was conducted to describe the epidemiological profile of imported malaria 2001-2011 in Jiangsu Province, where use to be a major malaria endemic area in China. Most of imported malaria cases were acquired from African countries, young male adults with the main travel purpose of exported labors were majority of population of patients. *Plasmodium falciparum* accounted for more than 80% of the infections, and a certain proportion of patients weren't received early diagnosis and proper treatment. In recent years, the significant growth of investment to Africa and the large number of exported labors caused the increase of overseas imported cases, there is possible increasing risk of re-introduction of malaria to the country from imported cases. The web-based real time disease reporting system is core for surveillance, and the important of having an efficient response mechanism to deal with imported malaria is highlighted.

1279

A TIME-SERIES ANALYSIS OF MALARIA CONTROL AND ITS EFFECTS ON PEDIATRIC BLOOD TRANSFUSIONS IN RURAL ZAMBIA

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Malaria related mortality remains a serious burden in sub-Saharan Africa, particularly among children. Blood transfusions can reduce mortality among children with severe malarial anemia. There has been little research conducted to date to measure the impact of malaria control on the use of blood transfusions in health facilities. We report findings from a time series analysis of facility and patient record data from a rural referral hospital over an eight-year period (2000-2008). We use multivariate analyses with an auto-regression-moving-average model to assess relationships between the scale-up of malaria control and pediatric blood transfusions. We also investigate the association between malaria control scale-up and the use of blood transfusions in other patient wards. Our results show that in years when malaria control was scaled up there were 21.9 fewer pediatric blood transfusions per month as compared to years when before malaria control scale-up (95% CI 8.1-35.8; $p<0.01$), a 56% reduction. Pediatric admissions for severe malarial anemia declined over the same period. In the maternity ward, there were 1.1 additional blood transfusions per month during the years of malaria scale-up (95% CI 0.1-2.1; $p<0.05$) as compared to years before malaria scale-up. This study provides important evidence that malaria control can reduce pediatric admissions for severe malarial anemia and thereby lower the use of pediatric blood transfusions. Our findings also suggest that malaria control may provide indirect benefits to non-malaria patients through greater availability of blood resources.

1280

COMMUNITY BASED MALARIA ELIMINATION EFFORTS IN SOUTHERN ZAMBIA

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Progress in malaria control efforts in Zambia resulted in a drop in malaria *parasitemia* in children under five from 22% in 2006 to 16% in 2010. This success, however, is not uniformly distributed with certain areas of Zambia reporting resurgence in malaria cases while other areas, mainly Lusaka and Southern Provinces have reached sufficiently low levels of malaria transmission to warrant an in-country push towards malaria elimination. Given this level of progress, the Zambian Ministry of Health set a goal of achieving malaria elimination in at least five areas within the country by 2015. This goal warrants the establishment of a robust malaria surveillance system with a high level of sensitivity to detect malaria infections at community level. The existing passive malaria surveillance

system, which detects malaria cases at formal health facilities, has been enhanced by leveraging volunteer community health worker networks to detect hotspots of malaria transmission through follow up and screening of households in proximity of identified index cases. These enhancements have been termed "Step 3" and constitute the final stage of an innovative three-step sequence designed to measure the progress, and move towards malaria elimination. Through Step 3, over 450 community health workers from four districts of Southern Province received a 4-day refresher training in aspects of clinical presentation, testing using rapid diagnostic tests and treatment of uncomplicated malaria according to current Ministry of Health policy. Further trainings were conducted in 2013 and will substantially increase the number of community health workers and the geographical area being considered for possible malaria elimination. Through Step 3, over 20,000 RDTs have been administered. Average positivity rate during community testing has been 11.87. Reporting completeness from community health workers each month has averaged approximately 90%. Initial results show this program increases the sensitivity and timeliness of malaria surveillance such that malaria infections previously undetected by the routine passive surveillance system are now being identified and treated. Data are being monitored by district personnel for hotspot activity to guide interventions. This presentation will highlight the efforts, successes and challenges faced during the implementation of this program.

1281

MALARIA EPIDEMIC SURVEILLANCE SITES IN THE SENEGAL RIVER VALLEY, 2008-2012

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As malaria transmission falls in a region, residents may not have sufficient exposure to infective bites to maintain immunity, and it may become epidemic-prone. The Senegal River Valley is epidemic-prone and experienced malaria epidemics in the 1990s. Artemisinin-based combination therapy (ACT) was introduced in 2007, and rapid diagnostic tests (RDTs) in 2008. Mass distribution of insecticide treated nets for children under 5 years took place nationwide in 2009, and universal coverage distribution in 2011. In 2007, the Senegal National Malaria Control Program (NMCP) put in place eight epidemic surveillance sites at health posts in four districts in the Senegal River Valley. Using a standard spreadsheet, sites report the number of total consultations, suspected malaria cases, patients tested, and confirmed cases of malaria. Data quality was assessed with quarterly onsite supervision. After 2008, diagnostic effort (cases tested/cases suspected) consistently surpassed 95% and was 100% annually in half the sites, with near absolute promptness and completeness. Transmission was highly seasonal, with 80% of cases occurring from August to November, with 60% in September and October. The southernmost site was found to be inconsistent with the epidemiologic profile of the others, with a mean annual incidence of symptomatic malaria of 93/1000 over the five years. In the remaining sites, mean annual incidence of symptomatic malaria from 2009-2012 was 1.7/1000; 0.2/1000 in children under 5, 0.6/1000 in pregnant women, and 2.0/1000 in the remainder of the population. Less than 10% of all consultations were suspected malaria, and RDT positivity rate among those tested was 17%. An investigation of the cause of high incidence in the southernmost site was conducted in 2010, but no epidemics occurred during the surveillance period. Given the low incidence and simultaneous scale-up of diagnostics, it was not possible to detect the impact of vector control interventions. Epidemic surveillance sites have performed well in Senegal and increased the districts' capacity in surveillance. The NMCP continues to add sites as transmission decreases, with the goal of detecting and responding to epidemics within two weeks.

1282

WHERE TO START IN ELIMINATING AN INFECTIOUS AGENT?

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Programs that have been successful in eliminating a disease from a large area have generally concluded that they should have focused their efforts earlier in the places with the highest transmission. These remained a threat after transmission was interrupted elsewhere, leading to the need to maintain potentially expensive surveillance activities in peripheral areas after the disease has been eliminated from them. We use a simple mathematical model of cost effectiveness to consider in which order to eliminate transmission in two connected zones, given that this is technically feasible, but that resource constraints allow an attack phase in only one zone at a time. We make simple sets of assumptions about receptivity, vulnerability, and costs. Irrespective of transmission level, disease burden is minimised by attacking the higher transmission site first. In low transmission areas, costs are minimised by attacking the higher transmission site first, while if both zones have initially very high transmission, costs are minimised by attacking the lower transmission site first. These results are scale-invariant (implying the units might be small patches, villages, districts, or countries), and can be generalized to any number of units. Considerations of equity and efficiency both argue against elimination strategies that concentrate resources in areas with the lowest transmission.

1283

THE ROLES OF VECTOR CONTROL IN ENABLING MALARIA ELIMINATION CAMPAIGNS IN VARYING TRANSMISSION SETTINGS

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Planning malaria elimination programs requires an understanding of local transmission dynamics and intensities, the local vectors species, their ecologies and behaviors. Computational models of malaria transmission can then be used to simulate the effects of different combinations, timings, and durations of vector control interventions. The EMOD model was used to simulate transmission dynamics for sites in Nigeria, Kenya, Tanzania, Zambia, and the Solomon Islands where a wide range of transmission intensities are exhibited that vary by vector species with different behaviors and population dynamics. Interventions tested in silico included insecticide-treated nets, indoor residual spraying, long-lasting larvicides, spatial repellents, and attractive toxic sugar baits. The impact of timing and duration of spray and larvicide rounds were examined for impact on the human parasite reservoir during the dry season, which affected the ability of dry season drug distribution rounds to eliminate transmission. The impact of vector control interventions depended on both the baseline transmission intensity and the behavior and ecology of each local vector species, with the species composition changing in simulation as interventions were applied. These computational results demonstrate the importance of field entomological data and understanding the transmission context for elimination programs.

1284

IDENTIFYING CHILDHOOD MALARIA HOTSPOTS USING MATERNAL SEROLOGICAL RESPONSES IN ENTEBBE, UGANDA, AN AREA OF HIGH MALARIA ENDEMICITY

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Identifying populations with the highest malaria risk can be a valuable preliminary stage in directing targeted malaria control and elimination programmes. Improving malaria surveillance in regions where malaria burden is greatest is undoubtedly essential. We hypothesised that serological markers in pregnancy can be used to identify spatial variation in childhood malaria transmission in highly endemic regions. In a randomised trial on anthelminthic use in pregnancy [ISRCTN32849447] 2,507 women were enrolled between April 2003 and November 2005, and 2,345 live births accumulated. Participants' addresses were geo-referenced using a handheld global position system (GPS). Maternal blood was collected at delivery and an enzyme immunoassay (EIA) was used to detect total IgG antibody concentrations (µg/ml) to Apical Membrane Antigen-1 (AMA-1) and Merozoite Surface Protein-1 (MSP-1). Childhood malaria episodes from birth to two years were recorded prospectively, and annual blood samples examined for asymptomatic parasitaemia. Hotspots of malaria transmission were identified by determining spatial patterns in the incidence of childhood malaria (Incidence rate/100pys (95% CI) IR=47.4, (45.3-49.5)), the prevalence of childhood asymptomatic parasitaemia determined by microscopy (10.4%, 95% CI: 8.2-12.6), and maternal levels of AMA-1 (mean $\log_{10}$=6.26, 95% CI: 6.19-6.34) and MSP-1 (mean $\log_{10}$=6.65, 95% CI: 6.58-6.71) antibodies, respectively. Two consistent hotspots were identified, and hotspots of maternal antimalarial responses to AMA-1 and MSP-1 overlapped hotspots of childhood clinical and asymptomatic malaria. Serological markers in pregnancy might be useful in identifying spatial variation in childhood malaria transmission at micro-geographic levels in highly endemic regions. Simple descriptive mapping using routine data collected at maternal and child health units, to instantly analyse epidemiological data, could be a cost-effective operational tool to detect hotspots of malaria and support planning and implementation of control activities.

1285

A MODEL FOR DISTRIBUTION OF THE CRYOPRESERVED PFSPZ VACCINE FOR FOCAL ELIMINATION OF MALARIA

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The PfSPZ Vaccine targeting *Plasmodium falciparum* (Pf) comprises attenuated, cryopreserved sporozoites that are stored and distributed through a liquid nitrogen (LN2) vapor phase (LNVP) cold chain using LNVP dry shippers. The vaccine is currently being evaluated in clinical trials in the U.S., Europe, and Africa. LN2 or LNVP cold chains are in common use in veterinary medicine for vaccines and artificial insemination. In human medicine LNVP storage is common, but LNVP cold chains are used on a smaller scale, principally for *in vitro* fertilization, regenerative medicine and anti-cancer vaccines. There are multiple advantages to using a LNVP cold chain, including independence from electricity. We recently reported on modeling the use of the LNVP cold chain for distribution of the PfSPZ Vaccine for use in the Expanded Program for Immunization (EPI). Using Tanzania as the example, the cost of distributing this vaccine was determined to be no different from that of distributing any newly introduced vaccine through the EPI. However, we are now aiming for use of the PfSPZ Vaccine in mass-administration to all age groups in campaigns targeting elimination of Pf malaria. For countrywide coverage, a new distribution model that incorporates a rolling series of focal campaigns based on zones, each of which utilizes a zonal storage hub, and delivery directly to immunization centers, has been developed. Zonal boundaries are defined by considerations of population density, geography, infrastructure and accessibility, and each is activated in a sequence determined by economics and seasonality of malaria transmission. We have applied this model to the distribution logistics of a 3-dose regimen of PfSPZ Vaccine for Pf elimination in Tanzania: here the model comprises 9 zones, each active for 4 months. Defining components of the distribution model are the number of doses/cryovial, the holding time and capacity of the LNVP dry shippers, the volume of, and rate of production of LN2, and the LN2 production equipment. These components can be modified according to specific zonal requirements. The model for complete population coverage for Tanzania will be presented.

1286

PERFORMANCE OF A FIELD-STABLE LAMP MALARIA KIT IN THE DETECTION OF ASYMPTOMATIC CARRIERS IN ENDEMIC AREAS OF CAMBODIA, ZANZIBAR, SWAZILAND AND COLOMBIA

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The ability to detect asymptomatic infections at a field level will be fundamental to the success of malaria elimination strategies. This requires highly sensitive screening tests close enough to the community to enable rapid treatment. Very low parasite density infections can be detected by molecular methods such as PCR; however, these techniques require considerable training and are restricted to reference laboratories. A new field-stable CE-marked diagnostic kit for malaria based on loop-mediated isothermal DNA amplification (LAMP) is now commercially available. This LAMP kit targets mitochondrial DNA of all *Plasmodium* (Pan LAMP) or of *Plasmodium falciparum* (Pf LAMP) parasites and is able to detect down to 1 parasite/µl of blood in less than 40 minutes. This assay is not only faster than PCR, but also requires minimal processing and instrumentation, and allows test reading with the naked eye. Compared to nested PCR and using samples from febrile patients, sensitivity and specificity for Pan LAMP were <97.0% and <99.2% respectively, and for Pf LAMP <93.3% and <85%, respectively. In order to evaluate the feasibility of this LAMP kit as a tool for the detection of asymptomatic malaria, dried blood spots from volunteers in endemic areas of Zanzibar, Cambodia, Swaziland and Colombia were collected. DNA extracted by Chelex-100 or Instagene reagent was used for amplification with Pan LAMP. Nested-PCR or nested-real-time-PCR were used as reference standards. In Cambodia, based on 516 samples, sensitivity and specificity of Pan LAMP were 86.4%(95%CI:76.6-92.7) and 93.3%(95%CI:90.5-95.4) respectively while on 465 samples from Zanzibar, sensitivity and specificity were 90.7%(95%CI:78.9-96.5) and 100%(95%CI:98.8-100) respectively. In Swaziland, 921 samples have been tested by LAMP and a positivity rate of 2.7% was observed. Nested-PCR results from these samples are pending. Sample collection in Colombia is ongoing and results will be available soon. Although LAMP testing was performed in reference laboratories, previous studies have demonstrated that the same performance can be

achieved by technicians without previous training, working in simple laboratory space and with basic equipment. FIND and partners are currently working on the development of a high throughput LAMP assay with a simplified sample processing method suited to large-scale screening campaigns for malaria elimination.

1287

GEOGRAPHIC EFFECTS ON THE DESIGN OF A TRIAL FOR INTERRUPTING THE TRANSMISSION OF MALARIA ON A SMALL ISLAND

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A cluster-randomized (stepped-wedge) A hierarchical stepped-wedge is implemented in the design of a trial of the use of odour-baited traps to eliminate *P. falciparum* malaria from Rusinga Island, Lake Victoria, Kenya (SolarMal trial). Each of 4062 households to receive the intervention are grouped into clusters of approximately 50 households. Groups of nine clusters are combined into meta-clusters. One randomly selected cluster within a randomly metacluster is selected to receive the intervention each week. Hierachical randomization sequences ensured that the intervention is rolled out completely within one meta-cluster before moving on to the next randomly selected meta-cluster. A stochastic model of malaria transmission incorporating first-order community effects applied to the household geography and membership was used to measure the efficacy of the intervention at each time step. Bootstrapped confidence intervals derived from several hundred model runs were used to identify those sequences which produced narrow (± 5 % width) confidence intervals until at least the last two months of the rollout, i.e., designs with the most power to distinguish differences between the intervened and the not yet intervened groups. Results were heavily influenced by the local variations in population density, a direct effect of the physical geography of the island. Of the random sequences that met these critera, additional social constraints were applied, e.g., each meta-cluster must have an equal chance of being the first meta-cluster to receive the intervention; households within a given village must all receive the intervention within six months. Ultimately, a set fifty of the most powerful designs were presented to community representatives as alternatives, and the one to be implemented was drawn by lot.

1288

CHARACTERISTICS OF CHILDREN WITH ASYMPTOMATIC MALARIA PARASITEMIA IN A HIGH-TRANSMISSION SETTING OF MALAWI

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Use of molecular diagnostics such as polymerase chain reaction (PCR) has led to the recognition that the majority of prevalent malaria infections are asymptomatic, and modeling suggests they play an important role in malaria transmission. We present data on asymptomatic *parasitemia* (AP) from a cross-sectional survey of children aged 6-59 months in Malawi enrolled in a cohort study. A census of six villages found 1,667 age-eligible children, of whom 1200 (72%) met inclusion criteria and consented. Caregivers were questioned regarding the child's illness history over the previous two weeks and a finger-prick blood sample was taken for slide microscopy and PCR. In March-April 2012, 440 (37%) out of 1186 providing a blood sample had *parasitemia* by PCR. Among parasitemic children, 291/430 (68%) had not been ill in the previous two weeks; 88% were not ill at the time of blood collection; and 89% had axillary temperature <37.5 °C. Among children not ill in the past two weeks, factors related to AP in a multivariate log-binomial model, included: age (16% increased risk per year of age, p<0.0001), wealth status (45% decreased risk for those in the wealthiest quintile, p=0.0002), and sleeping under a bednet the previous night (31% decreased risk, p<0.0001). No measured characteristics of parasitemic children differed between those reporting illness and those not, except antimalarial use in the prior two weeks (28% among symptomatic and 0% among asymptomatic children). Among PCR-positive children with blood smear results (n=326), the proportion with submicroscopic *parasitemia* was 35%. Our results suggest that fever surveys would miss more than two-thirds of malaria infections among children 6-59 months, and mass screen and treat strategies using microscopy or rapid diagnostics with similar sensitivity would miss more than a third of infections. Our results suggest that mass screen and treat with more sensitive diagnostics or mass drug administration may be required to significantly reduce the parasite reservoir in areas of moderate to high malaria transmission.

1289

INITIAL IMPACT OF LONG LASTING INSECTICIDE TREATED MOSQUITO NETS ON MALARIA IN KARIMUI, PAPUA NEW GUINEA

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Malaria control activities in Karimui, a remote area in the highlands region of Papua New Guinea, have historically had limited success. For example, a national malaria control program involving indoor residual spraying and mass drug administration carried out in the 1960s reduced malaria prevalence in the general population from between 5-10% to 1% across PNG, except for Karimui where the pre-implementation prevalence rate remained unchanged despite exposure to program activities. Karimui is also relatively unique in PNG terms as reliable malaria prevalence data has been collected in the region at several intervals over the past 50 years. After decades of inactivity, the national malaria control program with support from the Global Fund to fight HIV/Aids Tuberculosis and Malaria commenced costless mass distribution of long lasting insecticide treated mosquito nets (LLINs) across PNG in 2006. Drawing on the existing evidence-base, this study aims to assess the initial impact of LLINs on malaria epidemiology in Karimui relative to previous malaria control activities in the region. A household survey (HHS) was conducted alongside an annual census update round in a Karimui-based Sentinel Site in 2011. The HHS included 255 randomly selected households from across the Sentinel site. A structured questionnaire examining household level LLIN ownership and use (among other things) was completed with the head of each participating household. A blood sample was also drawn from all consenting individuals aged 6 months or older residing in each randomly selected household (n=1135). The resulting dataset was in the final stages of cleaning at the time of drafting this abstract. Analyses will be completed by July 2013 and will include LLIN coverage and utilisation rates and malaria parasitaemia prevalence in the general population. These findings will be compared and contrasted with earlier malaria epidemiological data obtained from the Karimui region.

1290

ASSESSING THE BURDEN OF MALARIA IN LARGE CITIES OF TROPICAL AFRICA - USE OF MALARIA INDICATOR SURVEYS AND DATA FROM THE MALARIA ATLAS PROJECT

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This presentation will demonstrate a simple method using data from Malaria Indicator Surveys ("MIS files") and/or data from the web site of the Malaria Atlas Project ("MAP files") to measure the prevalence of malaria *parasitemia* among children living in large cities of tropical Africa and compare this to the prevalence among children living nearby. Geo-coordinates for each survey cluster (in the case of MIS files) or research site (in the case of MAP files) were used to determine the distance from the site to the center of the city. Geo-coordinates of any site within 25 km of the city center were entered into Google Earth to obtain a satellite image of the location and determine whether it was within the boundaries of the metropolis. Data from all sites within city boundaries were pooled together and compared to data from all sites outside of city boundaries but within 100 miles of the city center. Data from the Uganda 2009 MIS showed that the prevalence of malaria *parasitemia* among children 6 - 59 months of age living in Kampala was 93% (95% C.I.: 85% - 97%) less than among children living outside the city. Data from the Nigeria 2010 MIS showed that the prevalence of malaria *parasitemia* among children living in Lagos was 95% (95% C.I.: 82% - 99%) less than among children living outside of the city. The prevalence of malaria *parasitemia* among children living in 7 large African cities in malaria endemic areas ranged from less than 1% in Dar es Salaam to 7.9% in Monrovia and was between 72% and 95% lower than in nearby sites outside of these cities. The method provides for a practical way to use existing and easily accessible data to rigorously document the substantially lower burden of malaria in various large cities of tropical Africa.

1291

THE INTERACTION BETWEEN IRON DEFICIENCY ANEMIA AND MALARIA ON ADVERSE BIRTH OUTCOMES

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The World Health Organization recommends iron supplementation and malaria prophylaxis during pregnancy to reduce adverse birth outcomes. However, there are concerns that iron supplementation can increase the risk of malaria and iron deficiency has been shown to protect against malaria. It is currently unknown how iron deficiency anemia and malaria interact to influence birth outcomes such as low birthweight. We determined malariametric and iron deficiency parameters (ferritin, CRP, transferrin receptor) in 470 pregnant women attending antenatal clinics in a malaria-endemic region of Papua New Guinea at enrolment (mean 25 weeks gestation). Women were followed to delivery and birth outcomes including birthweight and gestational age were recorded. The prevalence of iron deficiency anemia was high in this population (70-87%) depending on iron marker and definition. Linear regression showed an inverse relationship between ferritin (a biomarker of body iron stores) and birthweight; for every two-fold increase in ferritin, mean birthweight decreased by 58g (95%CI: -98, -18; p = 0.023) i.e. the more iron replete the greater the birthweight. There was some evidence of effect modification by the presence of *Plasmodium spp.* infection whereby the relationship between ferritin and birthweight was stronger in aparasitemic women (-67; 95% CI: -120, -15; p = 0.004) compared to parasitemic women (-31; 95% CI: -93, 31; p = 0.3). There was no association between iron status and gestational age/pre-term birth. This study provides evidence that iron deficient women are giving birth to heavier babies than their iron replete counterparts in a malaria-endemic area. Results question the use of universal iron supplementation during pregnancy for the prevention of adverse birth outcomes in malaria-endemic regions. Further research is needed to understand the mechanisms behind the protective effects of iron deficiency on adverse birth outcomes.

1292

SUBMICROSOPIC *GAMETOCYTEMIA* AND MALARIA IN MALAWI: MOLECULAR IDENTIFICATION AND IMPLICATIONS FOR TRANSMISSION

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Asymptomatic *Plasmodium* parasite infections occur frequently in people where malaria is endemic, and, if gametocytemic, may represent a source of "silent" transmission that is not associated with malaria disease. Microscopy is often considered insufficient to detect gametocyte infections, which occur at low densities relative to asexual parasite stages. A novel, highly sensitive and specific reverse transcription polymerase chain reaction (RT-PCR) assay was recently created that uses 5 markers to distinguish developing and mature *P. falciparum* gametocytes from asexual stages at submicroscopic densities. We evaluated this assay using human blood samples collected during October 2012 in a cross-sectional, all-ages, household-level study of the International Center of Excellence for Malaria Research in Malawi. We aimed to define potential infectious reservoirs by assessing prevalence and predictors of submicroscopic gametocyte infection in three settings representing urban/low (Blantyre), semi-rural/mountainous (Thyolo), and rural/high (Chikhwawa) transmission. Of 2,795 people who were surveyed and sampled for *Plasmodium* microscopy, additional blood from a subset of 629 people was collected into RNAprotect for RT-PCR testing. Of these, 618 had thick smear microscopy readings. Asexual stage parasites were detected by microscopy in 9.3% (16 of 173), 3.2% (7 of 218), and 13.3% (30 of 227) of samples from Blantyre, Thyolo, and Chikhwawa, respectively. Gametocytes were also detected in one person in this subset (from Blantyre). The use of this new RT-PCR assay enabled us to identify a large number of additional submicroscopic gametocyte infections, many of which were asymptomatic in this cross-sectional sample. We present data on the predictors of submicroscopic *gametocytemia* and compare these results with microscopic and molecular results for asexual parasites. A better understanding of which humans may be unrecognized sources of parasite transmission is critical in order to enhance malaria interventions, particularly in areas approaching elimination.

1293

INCIDENCE AND FACTORS ASSOCIATED WITH CLINICAL MALARIA AMONG SCHOOLCHILDREN IN A HIGH MALARIA TRANSMISSION SETTING

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Although school aged children bare the highest burden of asymptomatic malaria infections in high transmission settings, little is known about the burden of the clinical disease in this age-group. To investigate

the incidence and factors associated with clinical malaria among schoolchildren in Tororo, Uganda, we studied 248 children aged 6-14 years and enrolled in the placebo arm of a randomized placebo controlled trial investigating the impact of intermittent preventive treatment on malaria morbidity and cognitive function. Clinical malaria was defined as *parasitemia* with either history of fever or axillary temperature of greater than or equal to 37.50C. All children were followed for one year and clinical malaria was assessed by active case detection. Of the 248 children enrolled, 243(98%) completed the one year follow up. At baseline, *parasitemia* was present in 71(32%) of the children and the Incidence of clinical malaria was 0.34 episodes/child/year after one year of follow up. Clinical malaria episodes differed significantly by age groups with a 51% (p- value=0.029) reduction in the odds of disease in the older children (11-14 years) compared to younger children. Children infected with helminths were more likely to get clinical malaria than children without infection (OR 1.6 p-value=0.034). Interestingly, no association was observed between being parasitemic at baseline and development of clinical malaria during follow up (OR 0.96 p-value 0.891).Malaria (both asymptomatic *parasitemia* and clinical episodes) is a big health problem among schoolchildren in a high transmission setting and children may benefit from interventions targeted at reducing the malaria burden in this age-group. Combining malaria interventions to the already existing helminth control interventions in schools may provide cost effective means of extending malaria control in school aged children. Finally, in resource limited settings, targeting malaria intervention to younger children may provide considerable benefit in reducing risk of malaria and missed school days.

1294

THE OBSERVED OPTIMAL TEMPERATURE FOR MALARIA TRANSMISSION AT 25˚C IS PRECIPITATION DEPENDENT

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According to the 2007 IPCC report, the distribution and magnitude of malaria will be influenced by climate change. Exactly how is still debated. Previous studies showed that the optimal temperature for malaria transmission is 25˚C. Two studies differed with respect to how they were validated. While Lunde et al based the validation on laboratory studies, Mordecai et al used field data which showed that the highest values of EIR was observed around 25˚C. Since EIR is not only dependent on temperature, among other the availability of breeding sites, there is a possibility that this observed optimum is a result of more breeding sites in areas with temperatures close to 25˚C. To investigate whether the observed R0 maximum at 25˚C is due to more breeding sites in areas with temperatures close to 25˚C we run a previously described model, OMaWa for 20 years; one simulation with no temperature perturbation, and one simulation where air and water temperatures were perturbed with 2˚C. From the simulations we calculate the monthly mean basic reproductive number, R0, and find the breeding site dependent optimal temperature for malaria transmission. We use a non-parametric local maximum smoothing to define the temperature at which malaria is most efficiently transmitted. We find that the breeding site dependent optimum temperature for malaria transmission under current climate is ~24˚C. With a two degree increase, the observable optimum temperature for malaria transmission increases by one degree C. The model suggest the optimal temperature for malaria transmission derived from field observations is dependent on the actual air and water temperatures. To understand how climate change, ignoring changes in socio-economic conditions and interventions, influence malaria transmission and other vector borne diseases, there is a need to document the life history of vectors in relation to temperature in the laboratory.

1295

SPATIO-TEMPORAL SURVEILLANCE MODELS FOR THE DETECTION OF ELEVATED MALARIA RISK IN ETHIOPIA AND ZAMBIA

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In many African countries malaria transmission is being effectively reduced by broadly applied malaria prevention measures. However, there remains a need to develop methods to rapidly detect and respond to short term increases in malaria transmission. Passive surveillance data transmitted by mobile phones can provide a platform for rapid data transmission from facilities to district, national and international actors. Surveillance systems operating at scale deliver large quantities of data that require improved methods of interpretation and visualization to quickly simplify content for decision-makers. Furthermore, commonly utilized epidemic detection algorithms in most instances fail to make use of the information contained in the spatial structure of the data. Recent computational advances in spatio-temporal risk modeling using Integrated Nested Laplace Approximation (INLA) allow models accounting for spatial and temporal auto-correlation to be rapidly fit and updated, transforming surveillance data into timely and useful mapped risk notifications. Using the INLA package in R, such models were fit with high predictive accuracy for a sentinel surveillance system in Ethiopia (1,528 facility-month reports over a 39 month period in 83 facilities) and on a national HMIS dataset in Zambia (22,227 facility-month reports over a 24 month period in 1,369 facilities). These models made it possible to accurately identify spatio-temporal clusters of increased risk. When excedence probabilities were set to less than one in 1,000 for Ethiopia and less than one in 10,000 for Zambia, signaling events occurred at relative risk levels of 1,2,3, and 4 in 121, 32, 13, and 4 facility-months in Ethiopia, and in 4,175, 931, 283, and 133 facility-months in Zambia, respectively. Thresholds can be varied in this framework to balance the desired sensitivity of detection and programs' operational capacity to investigate each event. Such approaches will be of high utility to decision makers by improving both the speed and sensitivity of detection of increased malaria transmission.

1296

MALARIA TRANSMISSION IN HOUSEHOLDS IN BLANTYRE, MALAWI

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Epidemiological methods focused on the home, such as indoor residual spraying and the use of insecticide-treated bed nets, have proven effective in reducing the burden of malaria infection. Insight into the transmission of malaria within households might offer new strategies for malaria interventions. In urban areas, where *Anopheles* mosquitoes are rarely detected, we hypothesized that infections within households would be highly related because they were due to a single exposure outside of the urban area. We examined the relatedness of malaria infections in children participating in a clinical trial to their mothers in the city of Blantyre, Malawi. Children were enrolled in the study when they had an episode of uncomplicated malaria. Their mothers were offered the opportunity to be

tested for malaria when they felt ill. Blood spots from all of the mothers' specimens were tested for malaria by real-time PCR. We analyzed 177 new episodes of malaria in mothers among 101 individuals. Infections from the mothers and the infections detected in their children were genotyped using six neutral, unlinked microsatellite markers. Unique parasite genotypes were compared between children and their mothers and the mean proportion of shared microsatellites for the mother-child pair within a household was compared to the mean proportion of microsatellites shared among each child to every mother outside of his or her household by two sample t-test. Intra-household infections were more genetically related than inter-household infections (mean shared alleles 61.7% vs. 28.2% respectively, p value <0.0001). The extent of allele sharing suggests that drug resistant and drug susceptible parasites are passed between adults and children. Infections within a household may originate from a common source or are passed between members. If an exposure during travel outside the home introduces infection into the household, bed nets or chemoprophylaxis with travel may limit spread of malaria infection in urban areas.

1297

USING INTEGRATED LAPLACE APPROXIMATIONS TO ESTIMATE MALARIA PREVALENCE

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Malaria transmission intensity affects almost all aspects of malaria epidemiology, including community prevalence, incidence, and total malaria mortality. The most commonly measured metric of malaria transmission is the parasite rate (PR): the proportion of individuals infected at a given point in time. Previously, models built to predict PR on a global scale (Gething et al 2010) utilised Bayesian hierarchical models fitted by Markov Chain Monte Carlo (MCMC) sampling. While these models have proven to be very useful, MCMC sampling methods become intractable in terms of both convergence and computational time when used on large data sets. This limits their utility for large-scale malaria risk mapping, particularly as the number of available PR survey data continues to grow rapidly. An alternative new framework using simplified integrated nested Laplace approximations (INLA) to compute posterior marginals (Rue et al 2008) provides a powerful and flexible alternative. Here we show that the statistical performance of the two methods for spatial prediction of PR across large areas (using West Africa as an example) is comparable in terms of predictive validation statistics. We then demonstrate the use of the INLA framework to fit larger, more complex models, which could not otherwise be fit using MCMC sampling but which provide substantial improvements in model accuracy.

1298

CREATION OF CONTINENTAL-SCALE, TEMPORALLY DYNAMIC DATASETS FROM REMOTELY SENSED IMAGERY FOR USE IN DISEASE MODELING

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The proliferation of remotely sensed data products enables improved characterization of variables known to influence vector disease ecology. However, contamination of imagery by cloud and other data quality issues means dynamic data are often aggregated to synoptic means, limiting their utility for analyzing change. We have built two dynamic data assemblies for quantifying temperature and vegetation conditions (a lagged proxy for moisture) in Africa for use in modeling malaria. A newly designed gap-filling algorithm was central to our approach as an adjustment for persistent cloud cover in equatorial regions. Our resulting datasets consist of monthly estimates for each parameter (2000-2012, 1km spatial resolution, for all of Africa) and represent a noteworthy improvement over synoptic climatic summaries (e.g., single layers such as mean annual temperature). We discuss the implications of these new datasets for analyzing patterns and causes of changing malaria prevalence through time.

1299

RECOMBINATION AND RESOLUTION: A NOTE ABOUT *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-3 ALPHA AS A MOLECULAR MARKER

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Parasite molecular markers can provide much needed data on *Plasmodium vivax* populations, but there have been few suitable markers identified and analyzed. One marker that has been used extensively is the gene encoding merozoite surface protein-3 alpha (MSP-3 alpha), a blood-stage antigen known to be highly variable. Here, we report the results of a study using an augmented sample of complete MSP-3 alpha gene sequences (n = 48) to analyze patterns of parasite diversity at this locus and assess its utility as a genetic marker. In addition to small study populations of Venezuelan (n = 10) and Thai (n = 17) clinical isolates, we sequenced *P. vivax* strains from a diverse range of geographic locations. Evidence of frequent and variable insertion-deletion mutations and recurrent recombination between MSP-3α haplotypes in all populations complicated the inference of genetic diversity patterns and reduced the phylogenetic signal at this locus. Comparison to results from the in silico simulation of a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) protocol commonly used found that PCR-RFLP haplotypes were not informative of a population's genetic diversity and that identical haplotypes could be produced from analogous bands. Therefore, we question the ability of the PCR-RFLP protocol to accurately recapitulate the complex patterns of MSP-3 alpha recombination and polymorphism observed from sequencing. Our data suggest that a high number of genetic differences at the MSP-3α locus may be segregating between isolates in all *P. vivax* populations. Thus, we must caution against associating MSP-3 alpha allelic diversity with *P. vivax* population diversity, as MSP-3 alpha variability was as high within both local populations studied as in the entire diverse, global sample. High diversity allows the identification and tracking of individual parasite clones through time and space, and we suggest that this may be the most informative implementation of MSP-3 alpha as a molecular marker.

1300

A COMPARISON OF THE GENETIC STRUCTURES AMONG *PLASMODIUM* POPULATIONS FROM AREAS WITH DIFFERENT TRANSMISSION INTENSITIES IN COLOMBIA

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Malaria low-transmission areas are of great interest because of their potential for elimination attempts. Under such conditions, the precise monitoring of infections, including their spatial connectivity (gene flow), is indispensable. In 2011 Colombia reported a total of 61,636 cases, 47% less than in 2010, which may indicate a decreasing trend. About 42% of these cases were reported in four states (departamentos): Chocó, Cordoba, Nariño, and Valle del Cauca (Valle). Some representative areas like Buenaventura, (Valle) reported 979 cases in 2012, whereas Tumaco (Nariño) and Tierralta (Cordoba) reported 1,475 and 7,482 respectively. Furthermore, these areas display differences in the relative importance of *Plasmodium falciparum* (Pf) and *P. vivax* (Pv): Valle Pv 90%, Tumaco Pv 6.9%, and Tierralta Pv 92%. We hypothesized that in Pv infections, given the presence of hypnozoites and the high prevalence of subclinical infections, we should expect higher levels of recombination and multiple infections (MOI) as compared to Pf. We also hypothesized widespread clonal expansions in Pf, due to the effect of strong selection on mutations

conferring resistance in the recent past. We analyzed a total of 120 samples including both Pv and Pf parasite samples collected from these populations using a set of physically linked and unlinked microsatellite *loci*. We found that levels of heterozygosity varied geographically in both parasites. The frequency of multiple infections (MOI) ranged from 10-20% in Pv and was about 10% in Pf. The relative low level of MOI in Pv indicates that most patients cleared up their previous infections likely due to the easy access to Primaquine. We also found strong linkage disequilibrium (clonal expansions) in both species. Thus, the pattern observed in *P. vivax* is indicative of ongoing reduced levels of recombination due to the parasite demography. Overall, the local ecology appears to explain the turnover of clones/clusters in both parasites at this spatial scale. These processes need to be taken into account when studying gene flow among malaria endemic areas.

1301

MODELING FOR MALARIA CONTROL AND ELIMINATION SCENARIO PLANNING: APPLICATION OF THE EPIDEMIOLOGICAL MODELING (EMOD) MALARIA DISEASE TRANSMISSION KERNEL TO COMMUNITY-BASED INTERVENTION DELIVERY IN SOUTHERN ZAMBIA

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In the context of ongoing mass-screen-and-treat (MSAT) campaigns in Southern Zambia, we present the results of simulations using the Epidemiological Modeling (EMOD) program for the purpose of identifying optimal intervention strategies at different levels of endemic transmission. The EMOD modeling platform provides geographically-specific, and mechanistic stochastic models of disease transmission simulations through the use of extensive and complex software modeling. Given known or assumed parameters relevant for malaria control and elimination scenario planning for Zambia, we explore the impact of seasonality on optimal campaign timing and frequency; the cost-effectiveness of different modes of distribution, e.g. mass drug administration (MDA); the role of increased distribution and utilization of vector-control measures; and the addition of drugs with enhanced gametocidal and/or prophylactic effects such as primaquine. As malaria control and elimination efforts progress, models that optimize the combination of prevention and treatment strategies for delivery at community level are important to guide a rational approaches to choice of interventions and delivery methods.

1302

FACTORS INFLUENCING URBAN MALARIA: A COMPARATIVE STUDY OF TWO COMMUNITIES IN THE ACCRA METROPOLIS IN GHANA

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As urban centres in Ghana continue to grow, the scale and impact of urban malaria is increasing. This study was carried out to compare the prevalence of malaria in two communities and how this may be affected by knowledge, attitudes, socioeconomic status and preventive practices of residents in two communities within the Accra metropolis. Giemsa-stained thick blood films were examined for malaria parasites in 400 people (200 each from townships with high and low urban status) from May to November 2009. Questionnaires were administered to determine and evaluate demographics of the participants. All participants lived within the two catchment areas, about 20 km apart. Average malaria prevalence among participants was 8.75%. Prevalence in Kaneshie (12%: p=0.032) was however higher than that of Airport West (5.5%). Illiteracy rate (17.5%), self-medication (81.5%) and the use of coils (21.0%) as a control mechanism was higher among residents of Kaneshie than Airport West. Most of the people (40%) in Kaneshie did not use any form of malaria control method. Insecticide spray was the most preferred malaria control mechanism by the Airport West residents (60.5%). Overall knowledge about malaria, employment status, housing conditions, level of overcrowding and the cost of treatment of malaria was better in Airport West than at Kaneshie. Malaria prevalence and factors influencing its transmission differs within communities in the same urban area. It is therefore essential to develop control and prevention strategies based on the needs of specific communities.

1303

INTERACTION OF MALARIA AND HELMINTH CO-INFECTIONS IN SYMPTOMATIC AND ASYMPTOMATIC CHILDREN IN SOUTHWEST NIGERIA

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Malaria and intestinal helminth infections are common tropical diseases. Little is understood about their interaction when they coexist. We investigated the effect of co-infection of helminth and *Plasmodium* infections. Asymptomatic school children (304) and febrile children (495) were recruited from selected primary schools and Adeoyo Hospital, Ibadan, Nigeria). Blood samples were used for haematocrit determination while Giemsa stained smears were used for malaria parasite screening by microscopy. Stool samples were used for helminth diagnosis done by Kato-Katz method. Among the school children, 142 (46.7%) were positive for malaria, 181 (59.5%) had helminth only (*Ascaris lumbricoides*, AL - 43.1%, *Trichuris trichiura* TT -2.3% and AL/TT - 14.1%), while 57 (18.8%) had co-infection of helminth and *Plasmodium*. Among the febrile children, 116 (23.4%) were positive for malaria, 45 (9.1%) for worms only (AL- 7.3%, TT- 0.2%, AL/TT- 1.4%, *Taenia* spp - 0.2%) while 16 (3.2%) had co-infection of malaria and helminth. Among asymptomatic children, *Plasmodium* infection was significantly reduced in helminth positive relative to helminth negative. The opposite was the case among febrile children. Anaemia was significantly higher in *Plasmodium* infection alone compared with those with helminth infection. A. lumbricoides is the most prevalent helminth. *Plasmodium* infection was negatively and positively associated with helminth infection in asymptomatic and febrile children respectively.

1304

IDENTIFICATION AND MOLECULAR DIAGNOSIS OF A TANDEM DUPLICATION OF THE *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN GENE IN MADAGASCAR

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Previous studies have shown Duffy-negative African individuals are resistant to *Plasmodium vivax* (Pv) infections. However, recent findings in Madagascar confirm that Pv is capable of Duffy-independent red cell invasion. Data analysis has shown that infections from each individual field isolate are comprised of multiple Pv strains. Additionally, our whole genome sequencing of numerous Pv field isolates from Madagascar has led to discovery of a tandem duplication of the parasite's Duffy binding protein (PvDBP). The overall goal of our studies is to identify factors that play a significant role in Duffy-independent *vivax* malaria. For this study samples were collected from regions of western Madagascar where we originally identified Pv infections in Duffy-negative people. All samples were first analyzed by a *Plasmodium* species PCR-based ligation detection reaction fluorescent microsphere assay (LDR-FMA) to diagnose infection by the four species causing human malaria in Madagascar. To evaluate

complexity of Pv infection we developed nested PCR assays targeting Pv apical membrane antigen 1 gene (PvAMA1) and the PvDBP duplication to verify presence of Pv in individual infections. From a total of 138 samples, 42 were LDR-FMA-positive for Pv. Of these 0 were PCR positive for nest 1 PvAMA1, and 0 for nest 1 PvDBP duplication. However, 15 were PCR positive for the nest 2 of PvAMA1, 9 for PvDBP, and 7 were positive for both PvAMA1 and PvDBP. These results suggest that detection of Pv by focus on single-copy sequence requires nested PCR. Additionally, as Pv infections are characterized by the presence of multiple strains, our results indicate that the strains carrying the PvDBP duplication were present in approximately 25% of infections. When a PvDBP duplication strain was present, it made up varying proportions of the overall infection.

1305

SUB-NATIONAL EVALUATION OF THE IMPACT OF MALARIA CONTROL PROGRAMS IN HUAMBO PROVINCE, ANGOLA

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In most sub-Saharan African countries, malaria control activities are carried out at sub-national levels. Nation-wide activities often start with small geographical areas and expand to other areas. Monitoring and measuring the impact of interventions carried out at sub-national scale is vital to scaling these programs up to the national level, and is not always possible using national-level household surveys such as the malaria indicator surveys and demographic health surveys. These surveys are typically powered to generate estimates at either national or alternatively, one level below national scale. In Angola, we used routine health facility data to measure the impact of indoor residual spraying (IRS), insecticide treated net (ITN), and case management campaigns for malaria control that were implemented in three of the 11 municipalities in the Province of Huambo, Angola. Routine health information system data showed that suspected malaria cases in all ages decreased from 160,487 in 2009 to 135,018 in 2011, while deaths in children under 5 years of age suspected to be caused by malaria decreased from 506 to 141 (72%) during the same time period in Huambo Province. Although IRS and case management training programs were solely implemented in the Municipality of Huambo, the largest decrease in suspected malaria cases and suspected malaria mortality occurred in two municipalities that received a mass ITN distribution campaign in April 2011. In these two municipalities, the peak monthly incidence of suspected malaria cases decreased from 6.2 cases per 1000 population per month to 0.35 cases per 1000 population per month from 2011 to 2012. These results show that health facility data may be useful in measuring the impact of malaria control programs, and suggest that while the interventions in case management training and IRS appear to be decreasing the burden of malaria in Huambo Province as a whole, mass ITN distribution may have had the biggest contribution to this decrease. Other factors, such as infrastructure improvements in the Province may also have contributed to reducing malaria burden.

1306

HIGH RESOLUTION MICROSATELLITE "META-*LOCI*" TO STUDY THE MICROEPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM*

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Genotyping information may be used to provide fine scale data on parasite transmission networks, especially in low transmission areas. Microsatellites can be easily amplified from field samples and can readily identify alleles from multiple strains present in the blood. However, when performing many pairwise comparisons between samples, the probability of numerous alleles matching between unrelated parasites due to chance is unacceptably high, even when homozygosity at each locus is relatively low (~0.2). To improve genetic resolution, we investigated a "meta-locus" approach, in which the haplotypes of genetically linked microsatellites, instead of individual microsatellites, were used to define each locus. We identified 27 additional microsatellites demonstrating variability located within 15kb (with the exception of one due to an absence of closer microsatellites) of 10 commonly used microsatellites (range1-4 per locus), and thus unlikely to recombine over a few generations. Multiplex nested PCR methods were developed to increase sensitivity while conserving DNA. These methods were 10-100x more sensitive than individual PCR reactions, amplifying dried blood spot (DBS) samples with parasite densities of 10-100 parasites / ul. Preliminary genotyping data obtained from 50 dried blood spot samples in Uganda demonstrates that discrimination is increased on average 3.8 fold at each locus, with homozygosity decreasing from a median of 14% (interquartile range of 10%-13.5%) to 4% (interquartile range of 3.65%-4.55%). A number of meta-*loci* had unique signatures for almost all samples tested, indicating that homozygosity may have been overestimated in the samples tested. These methods offer promise for obtaining highly discriminatory multilocus genotypes from field samples. Application of these methods to evaluate fine-scale population structure is in process. In particular, samples from pre-elimination areas are being evaluated to identify the source and spread of malaria infections to better target interventions.

1307

WEALTH STATUS AND DEMAND FOR MALARIA TREATMENT FROM PRIVATE SECTOR RETAILERS IN NIGERIA

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Although AMFm subsidies have increased the supply of artemisinin combination therapies to treat malaria, there is little access to reliable malaria diagnostics in Nigeria even though national policy calls for parasitological confirmation prior to treatment. In 2012, we conducted REMEDI, a pilot study of the acceptability of malaria rapid diagnostic tests (RDTs) among adult customers purchasing anti-malarials from retail pharmacy and proprietary and patent medicine vendors (PPMVs) in urban and peri-urban areas of Oyo State in Southwest Nigeria. Using the pilot study data, our analysis aims to (1) assess the representativeness of our sample compared to a national survey and (2) investigate differences in malaria-treatment seeking behavior, acceptability of RDTs, and treatment adherence by individuals of different wealth statuses. To enable external comparison, wealth indexes are constructed using principal components analysis of household assets measures collected in both REMEDI and the 2010 Malaria Indicators Survey (MIS). Indexes are then converted to quintile categories according to cutoff values defined by the MIS and used as the main predictive indicators in subsequent bivariate and multivariate regression analyses.A comparison of wealth quintiles between REMEDI and the MIS shows that the REMEDI sample is substantially wealthier than the national population and concentrated within the top two wealthier quintiles. Regression analyses indicate that individuals in the highest wealth quintile are significantly less likely to be recruited at a PPMV, but more likely to report having gone to a PPMV for the previous episode of suspected malaria. The wealthiest also paid somewhat less for their drugs. No differences in other health-seeking behaviors, types of anti-malarials purchased, or RDT-positivity was detected, but the wealthiest individuals were less likely to take the correct treatment (according to the RDT result) even though they were more likely to consult the treatment advice card given to them by the study nurse. While the wealthiest individuals were also more educated, acceptability and adherence to RDT results may be more problematic and require targeted intervention.

1308

DIFFERENCES IN THE EPIDEMIOLOGY OF MALARIA IN THE GAMBIA, SENEGAL AND MALI

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Wide-scale deployment of improved access and coverage with malaria control tools will reduce malaria transmission in highly endemic areas and may ultimately lead to the elimination of malaria as a major public health problem in West Africa. In order to monitor changes in malaria epidemiology related to policy, both cross-sectional and cohort study designs have been used to target representative samples of the local population in 3 West African Countries: The Gambia, Mali and Senegal. Two sites in Mali characterized by high and intense transmission (irrigated sahelian areas of Dioro and Sudan Savana areas of Dangassa) have been selected. In Senegal, the Thies site is urban with moderate seasonal transmission, whereas the Gambian site is rural and has achieved a significant reduction in the intensity of transmission. We report here the results of 2 cross-sectional surveys carried out in the rainy and dry season respectively at the Mali sites and one cross sectional survey performed in the rainy season at the sites of Gambia and Senegal. The prevalence of asymptomatic infection in all age groups included during the rainy season varies from 0.3% (4/1497) in Urban Thies, 3.4% in rural Gambissara (48/1401), 20.4% in irrigated site of Dioro (301/1479), to 42.6% (601/1412) in Sudan Savana of Dangassa. The prevalence of symptomatic malaria within 2 weeks period in the same cohort was 0% in Thies (N=1497), 1.8% in Gambissara (N=1401), 2.1% in Dioro (N=1479) and 9.4% (N=1412) in Dangassa. During the dry season (February-March) the prevalence of asymptomatic infection in the same cohort remained relatively high in Dangassa : 45.8% (N= 1153) and lower in Dioro 8.4%. These results shows the challenges in control in high endemic areas such as Dangassa. In addition to transmission patterns, the differences between these 4 sites may reflect the different levels of use and coverage with malaria intervention tools.

1309

WHO CRITERIA FOR SEVERE MALARIA IN IDENTIFYING SEVERE *VIVAX* MALARIA: PRELIMINARY DATA FROM A STUDY IN IQUITOS, PERU

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Vivax malaria is responsible for 90% of malaria cases in Peru. Severe *vivax* malaria is defined using the WHO criteria devised for *Plasmodium falciparum*, but may lead to misclassification in *vivax* malaria. We report preliminary findings from a case-control study for severe *vivax* malaria. The study is being conducted in Iquitos, in the Peruvian Amazon. Participants were PCR confirmed *P. vivax* mono-infection 5 to 65 years old. Cases were defined using the WHO severe malaria criteria. Controls were uncomplicated *vivax* malaria, two for each case. Criteria for critically ill malaria case included very severe anemia (hemoglobin <5 mg/dL), lung injury, shock, renal failure, admission to the ICU or cerebral malaria. All cases and controls provided informed consent and were treated by the Ministry of Health following local guidelines. Thirty cases and 59 controls were enrolled based mainly on clinical criteria. None of the subjects tested positive for dengue or leptospirosis. The main characteristic of cases was prostration (96%). Other characteristics at admission were severe anemia (n=2), seizures (n=1), coma (n=1), jaundice (n=2) and pulmonary alterations (n=3). After 24 hrs, when the laboratory results were available, 11 controls (19%) were re classified as cases due to total bilirubin > 2.5 mg/dL (n=9), glucose < 60 mg/dL, and hematocrit <21% (n=1). No subjects presented with altered renal laboratory parameters. Prostration was the only severity criteria in thirteen cases (32%). Neither of these cases met the criteria for critically ill patients as defined above. Seventeen subjects were critically ill. Prostrated-only cases, compared to controls, had no differences in hemoglobin, platelets or creatinine, but they had higher total bilirubin levels (76% vs 35%, p=0.008) and lower albumin levels (30% vs 93%, p<0.001). All but one subject were discharged from the hospital within three days. There is a need for a specific definition of severe *vivax* malaria. Prostration may be a sensitive but not specific criteria to identify severe and critically ill *vivax* cases.

1310

LOOKING FOR GOLD, FINDING MALARIA: 2012 MALARIA SURVEILLANCE IN GOLD MINERS' COMMUNITIES IN SURINAME

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Despite the marked reduction of malaria incidence in Suriname, malaria continues to affect the migrants' population (n= 15,000) involved in gold mining. Miners have been trained in the use of RDTs and treatment of uncomplicated malaria to provide services in their communities. Blood films are prepared for the quality control of all RDTs performed. They report to the Tourtonne laboratory (TL). The TL in the epicenter of the Brazilian gold miners' community in the city is the other component of malaria surveillance in gold miners' communities. The TL staff executes Active Case Detection Campaigns on a regular basis in gold mining areas. The surveillance data serves as the basis of this paper. In 2012, 321 cases were recorded, representing a decrease of 50.3% from the 646 recorded in 2011. *Plasmodium falciparum*, *P. vivax* and *P. malariae* were identified in 42.4%, 49.5% and 1.9% of cases respectively. 3.1% had a mixed infection. For 3.1% the species could not be determined. 259 (80.7%) cases were imported; the 62 autochthonous cases signify a reduction of 62.4% compared to the 165 reported in 2011. Of the autochthonous cases, 30 (55.6%) were acquired in the Lawa basin, 11 (20.4%) around the Lake, 6 (11.1%) in the Saramacca and 3 (5.6%) in the Marowijne basin. The Upper Marowijne had the lowest number of cases 1 (1.9%). The 62 cases were dispersed over 24 locations with 5 or less cases per location. 52.4% of the locations had only 1 malaria case in 2012. The mean prevalence measured during ACDs was 1.8%. The SPR was 5.9%, ABER 36.2% and API 5.2 per 1000. 98.4% of the infections occurred in Brazilians. 4 cases were reported in pregnant women. Increased access to diagnosis and treatment in the remote gold mining areas, ACD campaigns and the distribution of LLIN to the populations at risk in the gold mining areas appears have contributed to the steep decline in malaria cases. An increasing proportion of the malaria cases appear to be acquired in French Guiana. A regional approach is mandatory to reduce cross-border importation.

1311

THE IMPACT OF MALARIA CONTROL INTERVENTIONS IN ETHIOPIA, 2000-2012

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Malaria transmission in Ethiopia is unstable, usually limited to areas <2000m in altitude, and 57 million people are considered at risk. Periodic, widespread malaria epidemics with high mortality have been historical features. However, since 2004, Ethiopia rapidly scaled up effective malaria interventions including: artemisinin-based combination therapies (ACTs), indoor residual spraying, long lasting insecticidal nets (LLINs), and universal laboratory diagnosis by microscopy or rapid diagnostic tests (RDTs). In addition, the total number of public health facilities increased 5-fold and >34,000 additional health extension workers were trained in malaria case management and supplied with RDTs and ACTs, which significantly increased Ethiopia's outpatient and inpatient malaria care capacity. Multiple data sources including program, survey, and surveillance data were reviewed to assess the scale-up of interventions and its potential impact. Although no ACTs were available in Ethiopia prior to 2004, more than 4 million treatment doses have been distributed yearly since 2006, sufficient to treat the nation's reported *Plasmodium falciparum* cases. Laboratory confirmation of all suspected malaria cases with microscopy or RDTs increased from <25% in 2004 to 83% by 2012. According to the national Malaria Indicator Surveys, 55% of households owned at least one LLIN in 2011 and access to malaria diagnosis and treatment services within 24 hours of fever onset has increased from 15% in 2007 to 51% in 2011. These improvements coincided with a 28% decrease in mortality from 2005 to 2011 among children less than five years of age. In addition, widespread malaria outbreaks have decreased. In 2003, a large-scale malaria outbreak resulted in an estimated 25,000 malaria-related deaths among children less than five years of age affecting 211 districts. Since then, fewer than 12 districts per year have reported malaria outbreaks. The scale up of malaria control interventions, treatment of laboratory-confirmed malaria cases with ACTs, and health systems strengthening are associated with reductions in annual malaria deaths among children less than five years of age and the suppression of malaria epidemics in Ethiopia.

1312

GENETIC DIVERSITY OF *PLASMODIUM VIVAX* INFECTIONS IN A REMOTE FORESTED AREA OF CENTRAL VIETNAM

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Plasmodium vivax control is becoming increasingly important in Vietnam where the malaria burden has been drastically reduced and the government has now engaged into a malaria elimination program. Understanding *P. vivax* transmission dynamics is crucial for further improving elimination strategies; however this knowledge remains scarce in most endemic areas. We present the baseline data on the *P. vivax* population genetics in a remote area of Central Vietnam. Two hundred and forty five blood samples collected before treatment (day 0) in *P. vivax* patients were submitted to species-specific PCR for diagnosis confirmation. All *P. vivax* mono-infections were genotyped using 16 previously published microsatellites. The overall genetic diversity and structure of the *P. vivax* parasite population was determined and related changes in space, time and demographic indicators were analyzed. A total of 239 patients were confirmed to be *P. vivax* mono-infections and genotyped. Overall the *P. vivax* population displayed a high genetic diversity with an expected heterozigosity (He) of 0.70 and an average of 1.21 alleles/locus (ranging from 1 to 5 alleles/locus). Most of the infections were polyclonal (75.3%) with an average multiplicity of infection of 2.7 haplotypes/person. The risk of polyclonal infections ranged from 50% to 96% across villages, and was significantly higher in children compared to adults (73.8 % versus 26.2%). Moreover, compared to dry season, the risk of polyclonal infections was 9-fold higher during the rains. In conclusion, in this remote, forested area, the *P. vivax* population was highly diverse and polyclonal, indicating substantial ongoing transmission; interrupting it may require additional and new interventions to those currently deployed.

1313

SEQUENCING OF *PLASMODIUM FALCIPARUM* LIVER STAGE CD8 T CELL ANTIGENS TO IDENTIFY VACCINE CANDIDATES

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CD8 T cell mediated immunity is a critical arm of the immune response in radiation attenuated sporozoite (RAS) conferred protection against malaria. When parasite development is halted inside hepatocytes, malaria peptides are presented on the surface of infected hepatocytes through MHC Class I receptors for presentation to CD8 T cells. Characterizing *Plasmodium falciparum* (*Pf*) peptides presented on the surface of infected hepatocytes holds promise to identify novel liver stage antigens as vaccine candidates. Here we report that by establishing an *in vitro* system of liver stage schizont culture and utilizing state-of-the-art mass spectrometry approaches we have successfully identified *Pf* peptides expressed on human primary hepatocytes from various donors at 48- or 96-hrs after sporozoite inoculation. From these samples we were able to identify immunogenic *Pf* liver stage peptides that match several HLA supertypes from primary human hepatocytes. By continuing our screening process we will work to identify the full repertoire of *Pf* liver stage antigens as a pathway to accelerate pre-erythrocytic antigen discovery.

1314

PLASMODIUM ALVEOLIN 5 IS ESSENTIAL FOR THE NORMAL FORMATION OF INNER MEMBRANE COMPLEX OF OOKINETES

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Malaria parasites undergo multiple developmental stages and adopt a range of cell shapes, including both motile and non-motile forms. While the motility requires the motor complex associated with the plasma membrane of elongated shape, it remains an open question whether the cell shape itself is required for the differentiation to next developmental stage. All invasive stage parasites have submembranous flattened vesicle packed into continuous layer, called inner membrane complex (IMC), supporting the plasma membrane. We found that ALV5, a member of *Plasmodium* alveolin, is essential for the normal formation of IMC of ookinetes using knocking down of ALV5 in mosquito stage. ALV5-deficiency resulted in the developmental arrest at a point of apical end formation from remnant zygote and the extremely low invasion ability to mosquito midgut due to its lost motility. However, intrahemocoel injection of arrested parasites resulted in normal development of sporogonic stage. These findings clearly indicate that the molecular machinery for differentiation is developed independently of cell shape of parasites.

1315

RALP1, A RHOPTRY-NECK, ERYTHROCYTE-BINDING PROTEIN OF *PLASMODIUM FALCIPARUM* MEROZOITES, IS A NOVEL VACCINE CANDIDATE ANTIGEN

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Erythrocyte invasion by merozoites is an obligatory stage in *Plasmodium* infection and is essential to malaria disease progression. Proteins in the apical organelles of the merozoites mediate invasion into the erythrocytes, and are potential malaria vaccine candidates. The rhoptry-associated, leucine zipper-like protein 1 (RALP1) in *P. falciparum* was previously found to be specifically expressed in schizont stages and localized to the rhoptry of merozoites based on immunofluorescence assay (IFA). Also, RALP1 has been refractory to gene knockout attempts, suggesting that it is essential for blood stage parasite survival. These characteristics suggest that RALP1 is a potential blood-stage vaccine candidate antigen, and here we aimed to assess its potential in this regard. Antibodies were raised against recombinant RALP1 proteins synthesized using the wheat germ cell-free system. Immunoelectron microscopy demonstrated for the first time that RALP1 is a rhoptry neck protein of the merozoites. Moreover, our IFA data showed that RALP1 translocates from the rhoptry neck to the moving junction during merozoite invasion. Growth and invasion inhibition assays revealed that anti-RALP1 antibodies inhibit invasion of erythrocytes by merozoites. Erythrocyte binding assays revealed that RALP1 possesses an erythrocyte binding epitope in the C-terminal region, suggesting that RALP1 represents a new *P. falciparum* erythrocyte binding protein. Human sera collected from malaria endemic areas in Thailand and Mali recognized this protein. Overall, our findings indicate that RALP1 is a rhoptry-neck erythrocyte-binding protein, and that it merits additional evaluation as a *P. falciparum* blood-stage vaccine candidate.

1316

NOVEL MOLECULE THAT IS SPECIFICALLY EXPRESSED ON MALE GAMETOCYTE AND MICROGAMETE HAS POTENTIAL ROLE ON EXFLAGELLATION

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Plasmodium transmission via mosquitoes required sexual stage parasite development and fertilization in mosquito midguts. After ingestion of gametocytes by mosquitoes, fertilization occurs to form zygotes, which develop into invasive forms, ookinetes. These transmission events occur rapidly within 24 hours after blood meals and many molecules are supposed to be involved. Nevertheless, little is known about molecular mechanisms how parasites transmit to mosquitoes. Previously, we reported a novel male specific protein, designated PyGM75, that is expressed in both gametocyte and gamete stage of *Plasmodium yoelii*. In this study, the subsellular localization of PyGM75 in male gametocytes and gametes were examined by immune-electron microscopy. It is revealed that PyGM75 is localized to the electron dense organelles, named osmiophilic bodies of male gametocytes, then transported to the surface of microgametes. Next, we produced pygm75 gene disrupted parasites to elucidate its function. Pygm75 disrupted parasites can normally differentiate into male and female gametocytes. However, these parasites were drastically impaired the exflagellation ability, therefore they could not form oocysts in the mosquito midguts. Further electron microscopic analysis demonstrated that osmiophilic bodies were disappeared from pygm75 disrupted male gametocytes. These results indicate that PyGM75 plays a crucial role in microgametes formation prior to fertilization.

1317

CONTRASTING ROLES FOR PFACS5 AND PFACS9 IN THE EXPANDED *PLASMODIUM FALCIPARUM* ACYL CO-A SYNTHETASE GENE FAMILY

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The remarkable plasticity of the *Plasmodium falciparum* genome allows for adaptation in response to selective pressures and challenges efforts to combat this important human pathogen. Evidence of the adaptive nature of this genome includes the expansion and recent positive selection of the acyl Co-A synthetase (ACS) gene family, which includes four orthologs predicted to activate exogenous fatty acids and play important roles in fatty acid scavenging as well as nine paralogs with unknown function. The evolutionary and functional significance for the expansion of the PfACS9 ortholog to nine paralogs, including PfACS5, is unknown, and we sought to functionally characterize these molecules to understand their biological role in the parasite. We therefore generated parasites with conditional knockdown of PfACS5 and PfACS9, which significantly reduced protein abundance, but led to no difference in intra-erythrocytic parasite growth, under either normal or restricted fatty acid growth conditions. To explore possible neofunctionalization, HA-tagged lines of PfACS5 and PfACS9 were characterized for timing of expression, subcellular localization, and interacting partners. We observed differential localization for these proteins using immunofluorescence assays. Unlike ACS9, ACS5 was clearly exported to the red blood cell cytosol and membrane periphery. Western blots of parasite lysate from subcellular fractions support this differential localization, and show peak expression at 30-36 hours post-

invasion (hpi) for PfACS5 and 38-44 hpi for PfACS9. Exploration of the PfACS interactome through pull down assays further supports distinct functions for these enzymes. We hypothesize that the expansion and recent positive selection of the PfACS gene family are the consequence of metabolic pressures driving parasite evolution, and characterization of this family may identify metabolic chokepoints and potential targets for novel antimalarials.

1318

EFFECTS OF RBC STORAGE CONDITIONS ON *PLASMODIUM FALCIPARUM* INVASION OF RBCS *IN VITRO*

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The *in vitro* culture of *Plasmodium falciparum* in red blood cells (RBCs) is essential to studying the molecular and cell biology of the parasite, however, culture methodologies differ between laboratories. One type of variability arises from RBC source and storage conditions. Recent protocols for standard parasite growth suggest collection and storage of RBCs in acid citrate dextrose (ACD), citrate phosphate dextrose (CPD), or citrate-phosphate-dextrose-adenine (CPDA). Most laboratories routinely culture *P. falciparum* in RBCs for up to 4 weeks after RBC collection, although it is known that freshly donated RBC sustain a higher *P. falciparum* growth rate. It is unknown what step of the *P. falciparum* intraerythrocytic life cycle is impacted by RBC storage. Studies on RBC storage for human clinical use in blood transfusions have revealed a relationship between RBC storage and transfusion complications. Current standards for blood banking involve using CPD for RBC collection, removing plasma and leukocytes, then storing RBCs in saline-adenine-glucose-mannitol (SAGM) for up to 42 days at 4°C. Many RBC storage lesions have been documented in these acidic medias, such as decreased deformability, decreased ATP, decreased 2,3-diphosphoglycerate (2,3-DPG), decreased intracellular potassium, increased intracellular NaCl, oxidative damage, lipid peroxidation, changes in membrane phospholipids, and vesiculation of membranes. Using flow cytometry based assays, we have separately examined the effect of RBC storage conditions and time in storage on overall parasite growth, merozoite invasion of RBCs, and merozoite production. We present results on the effects of storing RBCs after two, four, and six weeks in acid citrate dextrose (ACD) and citrate-phosphate-dextrose-adenine (CPDA), as well as in two other solutions known to maintain RBC integrity, RBC buffer (10 mM HEPES, 12 mM NaCl, 115 mM KCl, 5% BSA) and Alsever's solution.

1319

DE NOVO ASSEMBLY OF A FIELD ISOLATE GENOME REVEALS A NOVEL *PLASMODIUM VIVAX* ERYTHROCYTE-BINDING PROTEIN GENE

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Recent sequencing of *Plasmodium vivax* field isolates and monkey-adapted strains enabled characterization of SNPs throughout the genome. These analyses relied on mapping short reads onto the *P. vivax* reference genome generated from a monkey-adapted strain. Any locus deleted in this genome would be lacking in the reference sequence and missed in previous analyses. Here, we report de novo assembly of a *P. vivax* field isolate genome. Out of 2,857 assembled contigs, we identify 362 contigs each containing more than 5 kb of contiguous DNA sequences absent from the reference genome sequence. These novel *P. vivax* DNA sequences account for 3.8 million nucleotides and contain 792 predicted genes. Most of these contigs contain members of multigene families and likely originate from telomeric regions. Interestingly, we identify two contigs containing predicted protein coding genes similar to *Plasmodium* red blood cell invasion proteins. One gene encodes the reticulocyte-binding protein gene orthologous to P. cynomolgi RBP2e and P. *knowlesi* NBPXb. The second gene harbors all the hallmarks of a *Plasmodium* erythrocyte-binding protein but clusters separately from all known *Plasmodium* Duffy-binding protein genes. Additional analyses show that this gene is present in most *P. vivax* genomes and transcribed in blood-stage parasites. Our study complements previous genomic analyses and takes full advantage of sequence data to provide a comprehensive characterization of genetic variations in this important malaria parasite. Further analyses of the protein coding genes discovered have the potential to identify genes influencing key aspects of *P. vivax* biology, including novel mechanisms of human erythrocyte invasion.

1320

THE AUTOPHAGY PROTEIN PFATG7 IS THE ACTIVATING ENZYME OF THE *PLASMODIUM FALCIPARUM* PFATG8 LIPIDATION PATHWAY AND IS ESSENTIAL FOR NORMAL PARASITE GROWTH

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The *Plasmodium falciparum* genome encodes a limited number of putative autophagy genes, specifically the four genes involved in Atg8 lipidation, an essential step in formation of autophagosomes. In other eukaryotic systems, Atg8 lipidation requires the E1-type ligase Atg7, an E2-type ligase Atg3, and a cysteine protease Atg4. We have confirmed that these four putative *P. falciparum* ATG (PfATG) genes are transcribed during the parasite's erythrocytic stages. We hypothesize that these putative autophagy genes are the essential players of a functional Atg8 lipidation pathway in *P. falciparum.* Recent efforst have focused on dissecting the biochemistry of this pathway. We have genetically engineered parasites to allow for regulatable expression of the activating enzyme PfAtg7. Upon PfAtg7 attenuation, parasites exhibit slow growth in culture, indicating the essentiality of this enzyme for normal parasite growth. We have also modified the PfATG7 locus to introduce a C-terminal hemagglutinin (HA) tag. This has allowed us to immunoprecipitate native PfAtg7 enzyme to confirm its biochemical activity. In an *in vitro* conjugation assay combining native PfAtg7, ATP, and recombinant PfAtg8 followed by non-reducing SDS-PAGE conditions, we detect a PfAtg7-PfAtg8 thioester conjugate at approxiamately 150kDa using anti-PfAtg8. Upon reduction, the 150kDa conjugate is reduced to PfAtg7 and PfAtg8. This ability to form a thioester linkage with PfAtg8 provides evidence that PfAtg7 is in fact the activating enzyme of this pathway. As to translational implications of this research, specific inhibitors have been developed for E1-type ligases, such as the mammalian NEDD activating enzyme, and are currently in clinical trials as anticancer therapeutics. A similar strategy could be employed in the development of specific and selective PfAtg7 inhibitors. If successful, these inhibitors would represent a new class of antimalarials.

1321

DEVELOPMENT OF A NONRADIOACTIVE HETERODUPLEX TRACKING ASSAY TO MEASURE IN-HOST GENETIC DIVERSITY IN CLINICAL *PLASMODIUM VIVAX* INFECTIONS IN CAMBODIA

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Compared to *Plasmodium falciparum*, relatively less is known about the genetic complexity of *P. vivax* infections. Developing assays which can be used in malaria endemic countries to measure in-host diversity can further research into this neglected species. The Heteroduplex Tracking

Assay (HTA) sensitively detects different parasite variants simultaneously existing within an infected person, but relies on the generation of a radioactive probe, limiting its broad application. We developed a novel non-radioactive, fluorescently-labeled capillary electrophoresis-based HTA to measure multiplicity of infection based upon a region between the interspecies conserved blocks 5&6 of merozoite surface protein 1 (PvMSP-1). This new method relies on visualization of peaks that are detected as a heteroduplex formed by a fluorescently-labeled probe and patient-derived PCR amplicon migrate through a nondenaturing polymer, similar to the readout in microsatellite analysis. The new method was applied to *P. vivax* isolates from 25 patients from Anlong Veng, Cambodia. We found that the number of variants within individual persons ranged from 1 to 5 with a mean of 2.4 variants per sample. Virtual heterozygosity was high at 0.892, suggesting good allelic discrimination at the PvMSP-1 locus. In persons with recurrent *vivax* infections, we found reappearance of identical genetic variants in multiple recurrences, suggesting relapse rather than re-infection. We also found novel variants in these recurrent infections, suggesting that there remain minor variants in the initial *parasitemia* that are undetected, or that there are variants that do not emerge in the initial *parasitemia* but emerge in a relapse. These results are consistent with prior HTA studies from this region and reveal significant in-host malaria diversity in Southeast Asia. This new method will allow HTAs to used in malaria-endemic countries for clinical and research purposes.

1322

CHARACTERIZATION OF A TYPE 2C PROTEIN PHOSPHATASE IN *PLASMODIUM FALCIPARUM*

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Signaling pathway components, including kinases and phosphatases, have been a growing area of interest in the pursuit of novel antimalarials. Many have been identified as being more closely related to orthologues found in plants or other lower eukaryotes, making them attractive drug targets. In recent years, divergent kinases and phosphatases have been shown to play essential roles in both the human and mosquito stages of the parasite lifecycle. Type 2C protein phosphatases (PP2Cs) are serine/threonine phosphatases characterized by their magnesium dependence. While ten putative PP2Cs have been found in the *Plasmodium falciparum* genome, only one has been characterized, and is involved in the regulation of transcription and translation. We are investigating a PP2C, PfPP2C-1 (Pf11_0362), which diverges from a group of *Apicomplexa*n PP2Cs and shares closer homology with those of the plant *Arabidopsis thaliana*. Since *Arabidopsis* PP2Cs are critical for growth and stress responses, we are interested in the role of this late stage PP2C in *Plasmodium* schizogeny. We have successfully over-expressed PP2C-1 in *P. falciparum* parasite cultures using the mycobacteriophage recombination system, and found that it localizes to the cytoplasm. While we hypothesized that overexpression of PP2C-1 may lead to deregulation of its signaling pathway, these parasites showed no growth defect or phenotypic differences from wild type parasites. Functional significance of this protein is being assessed through gene knockout and knockdown strategies. We are using co-immunoprecipitation approaches to identify binding partners and potential signaling components of the protein. These studies may reveal hitherto unknown signaling pathways in *Plasmodium* schizogeny, and further define the critical role of PP2Cs in these parasites.

1323

GENOMIC STABILITY OF PFHRP2, PFHRP3 AND ITS FLANKING GENES OF *PLASMODIUM FALCIPARUM* WILD ISOLATES FROM THE PERUVIAN AMAZON REGION ADAPTED TO *IN VITRO* CULTURES DURING A YEAR

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Plasmodium falciparum parasites lacking pfhrp2 and/or pfhrp3 were restricted to laboratory strains which lose those genes during long period *in vitro* cultures. However, wild isolates lacking those genes were also found in the Peruvian Amazon Region as reported in 2010. It is hypothesized that these parasites could be losing both genes spontaneously by mitotic recombination in the asexual stage, similar to laboratory strains during long term cultures. The aim of this study is to assess the presence of *pfhrp2, pfhrp3* and its flanking genes as indicative of the subtelomeric regions stability from chromosomes 8 and 13. Six *P. falciparum* wild isolates were adapted and maintained on *in vitro* cultures during a one year period to simulate mitotic replications of the erythrocytic stages under no selective pressure forces. Previously, all samples (blood spots in filter paper) were characterized by PCR in order to confirm the initial *pfhrp2/pfhrp3* gene profiles: 2 (-/-), 1 (+/+), 2 (+/-) and 1 (-/+). Then, these isolates were maintained in cultures for 12 months and one aliquot per month were used to monitor the presence of these genes (pfhrp2, pfhrp3 and their flanking genes) and for the molecular genotyping using 3 genetic markers *(pfmsp1, pfmsp2* and *pfglurp)* and 14 microsatellites. All cultures maintained the original pfhrp2/pfhrp3 and their flanking genes profiles along the period of study (182.5 generations, 1 generation = 48 hrs. of intraerythocytic cycle). Only 1 isolate presented a switch between the *pfhrp3* profile from its filter paper sample (positive) and all its cultures (negative). The molecular genotyping showed the clonal nature of all the culture samples along the year and allows their monitoring along this period of time as quality control tool. In conclusion it was observed a genomic stability of *pfhrp2/pfhrp3* and their flanking genes in these isolates maintained during one-year *in vitro* culture. The switch in one isolate could be explained by the presence of more than one clone at the beginning of the study that was lost during the culture. Additionally, the classic genetic markers (*msp1, msp2* and *glurp*) were cost-effective and enough to determine the genotype of the isolates and as a quality control tool; but microsatellites brought wider information about those genotypes.

1324

PREVALENCE AND DISTRIBUTION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY AND MUTANT VARIANTS IN MALARIA PATIENTS FROM CAMBODIA

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Primaquine is a key component of current malaria control efforts in Southeast Asia. However, the safety of primaquine in patients with glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency remains a substantial safety concern given current diagnostic limitations in most malaria endemic areas. To better understand potential safety concerns and estimate risks for hemolysis that could result from widespread primaquine use, we evaluated the prevalence of G6PD deficiency, and attempted to characterize common G6PD variants in malaria endemic areas with known multidrug resistance along the Thai-Cambodian border.

We measured the prevalence of G6PD deficiency in a total of 1,188 patients in northern, western, central, and southern Cambodia from 2009 to 2012. Results from a qualitative G6PD fluorescence spot test were compared with a high resolution melting (HRM) real-time PCR method to detect G6PD variants. We developed the HRM assay to probe for single nucleotide polymorphisms (SNPs) associated with five of the most common G6PD variants previously reported in Southeast Asia: Viangchan, Mahidol, Canton, Jiangxi, and Chinese-5. Prevalence of qualitative G6PD deficiency among malaria patients was approximately 12%. HRM analysis revealed that the Viangchan variant, typically associated with moderate to severe (WHO Class II) deficiency, was most prevalent. The relatively high proportion of the population at risk with a mutation associated with moderate to severe G6PD deficiency cautions against widespread, unmonitored primaquine administration in Cambodia. In the absence of G6PD screening, and/or careful monitoring for potential hemolysis in unscreened patients, the risk of serious adverse events is high. Correlation with limited quantitative G6PD-deficiency data is currently underway, and will be presented to help estimate risk in this population, and better inform national malaria drug treatment policy in Cambodia.

1325

DELIVERY STRATEGIES FOR MASS CAMPAIGNS TO ACHIEVE UNIVERSAL COVERAGE WITH INSECTICIDE TREATED NETS: WHICH WORKS BEST? A MULTI-COUNTRY COMPARISON

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The use of insecticide treated nets (ITN) is widely recognized as one of the main interventions to prevent malaria. Mass distribution campaigns are the best approach to rapidly scale up ITN coverage. However, the best strategy to distribute ITN to households is still under debate. Data from 14 post campaign household surveys conducted in Nigeria, Ghana, South Sudan, Senegal and Uganda were merged. These campaigns used a variety of strategies such as stand-alone versus integrated distribution, fixed point versus house to house delivery and targeted or limited versus universal coverage ITN allocation. Survey design and data collection methods were similar across surveys, i.e. representative cross sectional household surveys with a two-stage cluster sampling design and a standard questionnaire. Analysis included 13,901 households and accounted for survey design and sampling probabilities. The main outcome indicators were the proportion of households that received at least one ITN from the campaign and the proportion of households reaching universal coverage on the survey day. None of the ITN campaigns increased the household coverage to the expected target of 80% or more households with sufficient ITN (one ITN for every two people or one ITN per sleeping place). There was no difference in campaign effectiveness comparing various strategies for distribution or delivery, providing that enough ITN are available. There were substantial discrepancies between the quantity of ITN distributed to households and the quantity needed in respect to people or sleeping places, independently of the indicator considered. The effectiveness of ITN campaigns does not depend on the strategy but rather on quality of implementation and ITN availability. Coverage achieved confirms that it is essential to complement mass campaigns with continuous distribution systems to achieve universal coverage targets.

1326

DOES A TORN LONG-LASTING INSECTICIDAL NET FAIL TO PROTECT CHILDREN FROM MALARIA PARASITEMIA? DATA FROM TWO CROSS-SECTIONAL SURVEYS IN WESTERN UGANDA

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Durability of long-lasting insecticidal nets (LLIN) is increasingly coming into focus since longer net survival is associated with significant public health savings. However, there is very little data on the extent to which damages to an LLIN limit its protective effect. In the context of malaria control efforts in Western Uganda, physical condition of nets was measured in a random sample of nets from two representative, cross-sectional surveys in July 2011 and October 2012, 18-30 months after mass distribution of LLIN in the area. From a total sample of 1,598 and 3,938 households 592 and 1,313 nets were assessed for physical integrity respectively using a proportionate hole index as recommended by WHO. Of these nets 818 (43%) had been used the previous night by children under five for whom data on malaria *parasitemia* were also obtained. Physical condition of LLIN was considered to be "good" when the total hole surface area on the net did not exceed 100 cm^2 and as "too torn" when more than 1000 cm^2. The proportion of the 818 nets in "good" condition decreased from 80% for nets less than 6 months old to 63% for nets 1-2 years old (p=0.02) and then stabilized around 50% suggesting that nets were discarded when too torn. Parasite rates in children 0-59 months of age decreased over time from 23% at the first survey to 15% (p=0.07) but did not vary significantly with physical condition (p=0.4) being 13.2% (95%CI 9.2-18.4) for "good" nets, 16.7% (11.7-32.4) for "damaged" nets and 18.3% (9.5-32.4) for nets "too torn". In a logistic regression model of *parasitemia* child age showed to be a significant determinant with an Odds-Ratio (OR) of 1.3 per each additional year (p=0.01) as well as district (p<0.005), current fever (OR 3.5, p<0.005) and second vs.first survey (OR 0.55, p=0.04). However, no increased risk of *parasitemia* was found for "too torn" nets (OR 1.0, p=0.9). These data suggest that, in the setting of Western Uganda, even seriously torn LLIN still provide sufficient protection for children and nets are discarded before they lose their protective effect.

1327

RISK FACTORS ASSOCIATED WITH MALARIA INCIDENCE AMONG YOUNG CHILDREN AND FEMALE *ANOPHELES* MOSQUITO COUNTS IN KOROGWE, TANZANIA

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Several studies conducted in Northeast Tanzania have documented a declining trend in malaria transmission beginning well before malaria interventions were scaled up. One explanation for the decline in malaria may be the changes in socioeconomic conditions associated with economic development, and in particular improvements in house construction materials. This analysis has two main objectives: (1) identify risk factors associated with malaria incidence among young children and (2) identify household and environmental factors associated with mosquito density in and around the home. A particular focus is paid to the housing construction materials as determinants in both analyses. For 435 children enrolled in larger trial of intermittent preventive treatment for malaria in infants in Tanga, North-eastern Tanzania, detailed information on their dwelling characteristics were collected. An index scale of housing structure

quality constructed via principal components analysis was converted to decile units for regression analysis. Ordered logistic regressions were used to predict risk factors for child malaria episodes (none, 1-2, or 3+ episodes) and negative binomial regressions were used to predict risk factors for average female anopheles mosquito counts collected in traps in and around the dwelling. Results suggest that, compared to children who reside in houses with better construction materials, residing in the worst type of house significantly increases the risk of malaria two- to three-fold, even when wealth and rural residence is controlled for. Having ceilings is associated with a significant reduction in female anopheles mosquito counts by nearly half, while having cattle around the house increases mosquito counts. In conclusion, these results corroborate findings from other studies of household and environmental risk factors that show associations between malaria risk to poor housing materials. Interventions to reduce the receptivity of an area or exposure via housing type could help to further reduce malaria transmission.

1328

SELECTION AND CHARACTERIZATION OF A NEW, NON-MELANISING, LINE OF *ANOPHELES GAMBIAE* REFRACTORY TO *PLASMODIUM FALCIPARUM*

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Anopheles gambiae is a principal vector of *Plasmodium falciparum* malaria in Africa. Some individual mosquitoes within a population are naturally refractory to infection. The only existing refractory line of *An. gambiae* (G3) melanises *P. falciparum* parasites, a refractory behaviour uncommon under natural transmission. Understanding common mechanisms of natural refractoriness could be used for development of transmission blocking vaccines or GMO vector strategies. We have selected a new, non-melanising, refractory line of *An. gambiae* from the outbred Keele line named GU-REF. GU-REF was selected for refractoriness to *P. falciparum* clone 3D7 over 11 generations of selection. At the same time, the GU-CON line was selected at random as a control for inbreeding effects. The refractory line was then tested for genotype specificity, parasite stages affected, timing of blood meal digestion after feeding on infected and uninfected blood, expression of candidate genes previously linked with refractoriness, and fitness parameters (costs of refractoriness). GU-REF mosquitoes exhibit a significantly lower infection prevalence compared to GU-CON and the parent Keele line. The refractory behaviour is not specific to the parasite clone (3D7) used for selection, in that refractoriness is seen to an unrelated parasite, HB3. The refractory mechanism affects the parasite stages before the early oocyst. GU-REF mosquitoes do not appear to exhibit fitness costs associated with refractoriness, as measured by fecundity. Protein digestion of the blood meal is slightly faster in GU-REF after an infectious blood-meal, compared to GU-CON. There is no difference in speed of digestion after a non-infected blood-meal. A new refractory line of *An. gambiae* refractory to infection with *P. falciparum* has been selected. The exact mechanism of refractoriness has not yet been characterised, but could involve the speed of blood-meal digestion or non-melanotic immune responses. The GU-REF line does not appear to have fitness costs associated with refractoriness.

1329

IMPACT OF INSECTICIDE TREATED WALL LINER ON SCHOOL ATTENDANCE IN RURAL WESTERN KENYA

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Results of a cluster randomized trial in rural western Kenya suggest a new form of malaria prevention, insecticide treated wall liner (ITWL) plus insecticide treated nets (ITNs), provide added benefit over that provided by ITN alone, reducing childhood malaria infection by 38% (95% confidence interval [CI], 23%-50%) overall and by 42% (95% CI=26%-55%) in children aged 5-11 years. Prior studies have shown that malaria adversely affects children's academic performance and school attendance. This supplemental study sought to determine whether children from the ITWL plus ITN (intervention) villages had reduced absenteeism when compared to children from the ITN alone (control) villages.We performed a retrospective analysis of attendance registers for children in standards (grades) 1 through 8 via comparison of school attendance in term 1 of the 2010 academic year (prior to ITWL) to term 3 (after ITWL). Data were available from 6 schools serving children from 8 of the 12 villages in the original trial. Using a multilevel mixed effects difference-in-differences regression model, we explored the effect of ITWL on attendance of pupils from intervention vs. control villages between terms 1 and 3. In order to adjust for registers with missing or incomplete recording of absences,mostly in the last weeks of each 13-week term, we choose to limit analysis to attendance from weeks 1-10 in each academic term. We adjusted for clustering by the inclusion of a village-level variable in the multilevel analysis; other covariates used in the analysis were categorical variables for standard, gender, village,and term. The resulting dataset had 1,126 observations, each representing the percentage of school days attended for one child for weeks 1-10 in a term. Overall recorded attendance averaged 90.1 percent (95% CI 88.9%-91.3%) over weeks 1-10 of term 1, so that recorded absenteeism averaged 9.9 percent. The interaction term in the regression showed that attendance improved (and absenteeism decreased) by 4.7 percentage points (95% CI 1.2%-8.1%) for children in intervention compared to control villages (p=0.008), representing a halving of recorded absences. The main limitation was our inability to confirm the overall accuracy of entries from existing school attendance registers. Nevertheless, these favorable preliminary results suggest a beneficial impact on school attendance from adding ITWL to ITN.

1330

PLASMODIUM FALCIPARUM GAMETOCYTES INFECTIVITY FROM POST ASAQ (ARTESUNATE AMODIAQUINE) TREATMENT PATIENTS SUPPLEMENTED WITH AZADIRACHTIN-ENRICHED NEEM EXTRACT

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Infectivity of malaria species to the mosquito vector has been investigated in the recent years by scientific community. Patients treated with artemisinin-based combination therapy, artesunate amodiaquine (ASAQ), against *Plasmodium falciparum* malaria produce fast clinical responses to

asexual stage of the parasite; but few data are available for sexual forms (gametocytes) responsible for the malaria transmission to human host to mosquito. In this study, *Plasmodium falciparum* gametocytes from naturally infected human after ASAQ 3-day treatment course in presence of azadirachtin-enriched neem (*Azadirachta indica*) extract were assessed for its infectivity to *Anopheles coluzzii*. *Anophèles coluzzii* females were membrane fed on gametocytaemic blood collected from patients after 3 day ASAQ treatment course and supplemented with azadirachtin-enriched neem (*Aza*) extract. Gametocytes infectivity was evaluated by assessing oocysts prevalence and intensity on mosquito midgut. Oocyst prevalence of 43% (CI_{95} 23-60) and oocyst intensity of 10.78 (CI_{95} 0.0-21.9) were still found after ASAQ treatment. However, a single dose of Aza added to gametocytaemic blood, completely block gametocytes infectivity at 60 ppm and reduce the oocyst prevalence to 98% at 50 ppm. This work demonstrated that after 3-day ASAQ treatment, patients are still able to maintain vector infection. But, single dose of Aza at 50 to 60 ppm will help in preventing mosquito infection and in blocking the malaria transmission.

1331

IMPACT OF COMMUNITY CHANGE AGENTS ON LLIN NORMS AND USE IN TANZANIA

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Since 2009, Tanzania has distributed 27 million LLINs. According to the 2011-12 THMIS, 91% of households now own at least one ITN and 68% of the population used an ITN the night before the survey. To achieve consistent universal net coverage, program planners need more information on the factors that influence net use in net owning households. Social norms are increasingly recognized as an important determinant of a range of health behaviors, although their role in malaria prevention is not understood. This study investigated the role of a Community Change Agent (CCA) program in affecting social norms related to use of long-lasting insecticidal nets. Since 2008, more than 1800 community members have been recruited and trained to become CCAs and to promote malaria awareness and discussion through community meetings, educational events and household visits. This study randomly recruited 1040 men and women living in the Lindi, Rukwa, and Mwanza regions of Tanzania to participate in a behavioral survey. Overall, 81% of respondents in net-owning households reported that everyone in the household slept under a net during the night before the survey. This outcome was significantly related to perceived social norms ($p<0.001$). Adjusting for background characteristics and number of household nets, universal use in a household increased from 57% in households where the respondent believed few or no households in the community used bed nets to 86% in households where the respondent believed that all households in the community used nets. In addition, controlling for background variables and the actual level of net use in the community, respondents who had interacted with the CCA were significantly more likely to believe that more households in their community used bed nets ($p=0.01$). The results of this study suggest that exposure to a community change agent indirectly affects net use through CCAs' effects on descriptive social norms.

1332

MALARIA CHEMOPROPHYLAXIS: WHY DON'T THE EXPERTS AGREE? AN INTERNATIONAL OPINION SURVEY

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The use of malaria chemoprophylaxis is central to pre-journey recommendations made to many of our travellers. We struggle to ensure that travellers understand the importance of strict adherence, and all too often medication is taken irregularly or just left at the bottom of the bag. One possible reason for the poor adherence is the wide variation in advice that is given to the public, with different expert groups recommending different drugs or no drugs for travellers with identical itineraries. We undertook a questionnaire survey of experts from around the world involved in the development of National Malaria Guidelines. There is a notable difference in the chemoprophylaxis recommendations in the National Guidelines produced by different jurisdictions. We aimed to find out what evidence is used by the different experts in the development of their guidelines; to better understand the reasons for the wide variation in chemoprophylaxis recommendations. The respondents were also asked what sort of evidence they would prefer to use if ideal data were available. We were unable to detect a marked difference in the evidence used by the different jurisdictions. This was true even when broken down to specific areas, such as that used for India. There are several possible reasons for the variation in recommendations despite using the same evidence base. It may be that we are interpreting the available data differently; it may be that the available data is too poor quality to be of any use; or it may be that we are just collating the wrong data. It may be that we have good data, but are drawn to alternative conclusions by other factors such as medico-legal risk and drug costs. It is difficult to know which recommendations will be proved correct, and the outcomes from the different policies will be fascinating to observe. However a there will need to be a coordinated effort to pool the traveller data from all countries to make sense of the results. The development of an agreed tool to standardise the weighting given to evidence types would give a clear rational for any chemoprophylaxis recommendations. This would, ultimately, improve chemoprophylaxis compliance amongst travellers.

1333

IDENTIFYING SUBPOPULATIONS LEAST LIKELY TO USE MOSQUITO NETS AFTER MASS DISTRIBUTION CAMPAIGNS: CASE OF KANO STATE, NIGERIA

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Insecticide-treated net (ITN) ownership and particularly use remain low in many malaria endemic countries in sub-Sahara Africa (SSA). With the shift from target group to universal coverage approaches there is need to ensure effective use of ITNs among all subgroups of the population. Identifying subgroups least likely to use ITNs could inform targeted messaging to improve overall coverage. This study aimed to identify the subgroups least likely to use ITNs after the mass distribution campaigns which took place in May and July 2009 in Kano State, Nigeria. The study used post-campaign evaluation survey data which was collected from October to November 2009. Individuals (3,056) living in households with at least one ITN and sleeping in the households the night before the survey visit (de facto population) were included in the analysis. Pearson Chi-square and Chi-square Automatic Interaction Detector (CHAID) regression were used to identify predictors of ITN use and the subgroups least likely to use ITNs. Eight covariate variables were included in the initial model. Five of these, including sex, age, wealth quintiles, education of the head of the household, and campaign distribution wave were used in the final

model as predicators of ITN use. Overall ITN use was 53% among all participants, and age and sex were good predictors of use. Males aged 15-25 years were the least likely to use ITNs, with a use rate of only 23%, while rates ranged from 46% to 62% among other subgroups. While further qualitative research may provide additional inside, these findings provide useful information for targeted awareness messaging during the mass distribution campaigns of ITN which are being implemented in several countries in SSA.

1334

EFFECT OF AGE OF ITN OWNED BY HOUSEHOLDS ON MALARIA PARASITE INFECTION AMONG CHILDREN UNDER FIVE YEARS OF AGE IN ANGOLA

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Insecticide treated nets (ITNs) are effective for malaria control and provide protection to individuals living in households that own them. ITNs are manufactured to have a long lasting protective effect; however, the effect of the age of ITNs in households on malaria *parasitemia* is not well documented. This study examined the association between the age of ITNs in households and malaria *parasitemia* among children under five years of age in Angola. ITNs that were obtained shortly before the survey may not protect children from malaria *parasitemia* because the infection may have happened before the acquisition of the net. Conversely, ITNs that were obtained a longer time ago may be less protective due to wear and tear of the net, or reduction in efficacy of the insecticide. We performed a multivariate logistic regression to assess the association between the age of ITNs in households and malaria *parasitemia* among children under five years of age using the 2011 Angola Malaria Indicator Survey. We adjusted for eight potential confounders: sex of child, age of child, mother's level of education, whether the household had been sprayed or not in the previous 12 months, household size, household wealth, area of residence, and malaria epidemiologic zones. Children from households that had owned ITNs for 2-6 months before the survey were significantly less likely to have malaria *parasitemia* compared to those from households without ITNs (OR = 0.28, 95% CI: 0.10-0.84). ITNs that had been owned for 1 month or less, or for more than 6 months, were not protective. These findings provide useful information, particularly when assessing the impact of ITN interventions on the reduction of malaria burden.

1335

MYANMAR ARTEMISININ RESISTANCE CONTAINMENT (MARC) SURVEY: MALARIA AWARENESS AND PREVENTION

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Despite anecdotal evidence of declining malaria transmission in some parts, Myanmar has the malaria burden in the Greater Mekong Sub-region. With the emergence of artemisinin resistance in the region, Myanmar is at the forefront of containing and ultimately eliminating artemisinin resistant parasites. In 2012, a malaria survey of households was conducted in the areas of known and suspected artemisinin resistance (Tier 1 and Tier 2) to serve as a baseline for the Myanmar Artemisinin Resistance Containment (MARC) efforts. The study domain included representative populations living in high to moderate malaria risk areas and utilized a multi-stage sampling approach stratified by Tier. Overall, 1992 household respondents were interviewed using standardized and pre-tested questionnaires in line with similar malaria surveys previously conducted in Cambodia and Thailand. Overall, 66.5% (95%CI 62.2 to 70.6) of household respondents understood "mosquito bites" as a mode for malaria transmission and 17.2% (95%CI 14.2 to 20.6) did not mention any transmission mode. Household coverage with at least one mosquito net was 97.5% (95%CI 95.1 to 98.7) and insecticide treated net (ITN) was 35.1% (95%CI 28.4 to 42.4). Lastly, 76.5% (95%CI 72.9 to 79.8) of all people (n = 9408) used a mosquito net the previous night and 15.9% (95%CI 12.4 to 20.3) slept under an ITN. General awareness of malaria was found to be modest; further efforts should be placed on improving community perceptions and behaviors for malaria prevention. Household coverage of ITN seemed insufficient to have an impact on reducing malaria transmission. Considering the high coverage and use of untreated mosquito nets, the national malaria prevention strategy should explore short to medium-term approaches to convert these untreated nets into ITNs and LLINs. For the longer term, demand-driven strategies should be in place to replace current untreated mosquito nets, building on the existing "net culture" in Myanmar.

1336

INSECTICIDE TREATED NET USE UNDER A COMPREHENSIVE DISTRIBUTION PROGRAM IN KENYA: SUCCESSES AND UNAVOIDABLE SHORTFALLS

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Insecticide treated nets (ITNs) have proven instrumental against holoendemic malaria. As distribution of ITNs throughout sub-Saharan Africa (SSA) is being scaled up, however, maintaining high levels of coverage over time will be instrumental to sustain current gains. We evaluated the impact of an ITN mass distribution campaign in early 2011to a rural Kenyan community along Lake Victoria. Surveyors collected data on ITN use both before and one year following this distribution. At both times, household representatives were asked to provide a complete accounting of ITNs within the home, including net locations and the ages and genders of people sleeping under them the previous night. Other data on household material possessions, education levels, occupations, and community group memberships were recorded. Patterns of ITN use before and following distribution were compared using spatial and multi-variable statistical methods. At the time of distribution, ~50% of residents reported sleeping under an ITN the previous night, a use rate that rose to 92% one year following mass distribution. However, ITN use varied by age and gender, following a similar pattern both pre- and post-distribution. After infancy, ITN use sharply declined until the late teen years when it began to rise again, plateauing at ~30 years of age. Prior to distribution, socio-economic factors such as parental education and occupation were associated with ITN use. Following distribution, ITN use was similar across social groups. Household factors such as ITN availability and sleeping arrangement negatively impacted use. Our results indicate that mass distribution of ITNs was effective in rapidly scaling up coverage. Free distribution of ITNs using a direct-to-household method can eliminate socio-economic and spatial heterogeneities in ITN possession and use. Age is an important factor in determining consistent ITN use, but problems of sleeping arrangement and ITN disappearance will present a challenge to effective intervention campaigns.

1337

COST EFFECTIVENESS OF INDOOR RESIDUAL SPRAYING IN NYANZA PROVINCE KENYA

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From its peak in 2004 to 2010, global malaria mortality fell by 32% from 1.82 to 1.23 million according to a 2012 Lancet paper by Murray et al. The expansion of indoor residual spraying (IRS) and insecticide treated nets (ITN) are considered important contributing interventions to this decrease. However, few empirical studies exist about the cost-effectiveness of IRS as a supplement to ITN, particularly in normal operational programs. We conducted a retrospective cost-effectiveness study of this use of IRS in 2010, combining data from two adjacent districts with perennial malaria transmission (greater Nyando and Rachuonyo) in Nyanza Province, Kenya. We assessed district-level costs by developing spreadsheet templates enumerating the categories of inputs (personnel, recurrent, capital), and the quantities and unit costs of each input within each category. We assessed quantities and unit costs through local and national key informants, consulting catalogs, and checking consistency (e.g. known productivity of spray operators and equipment per operator). We amortized capital inputs based on previous publications of the US President's Malaria Initiative (PMI), and computed cost per person in greater Nyando based on the Kenya census estimates. We estimated clinical malaria cases averted from trial previously published from Rachuonyo, and converted this to discounted life years gained (DLYG) by linking with other previous studies. IRS cost 229 Kenyan shillings (US $3.16) per person in the population, with shares of 22% for personnel, 69% for recurrent cost, and 10% for amortized capital. The breakdown for recurrent costs was 35% for vehicle rental, 27% for insecticide, 31% for personal protective equipment, and 7% for other. Per 100 person years, the combination of IRS and ITN compared to ITN alone reduced infections from 44 to 18, clinical cases from 27 to 9, and added 2.27 DLYG. These give cost-effectiveness ratios of $12 per malarial infection averted, $18 per clinical case averted, and $139 per discounted life year gained--a ratio substantially below Kenya's per capita GDP of $795 (a WHO threshold). Our cost per person covered was about half the median reported from 12 PMI countries ($6.94). While a more systematic addition of national and international overheads would increase costs somewhat, our analysis nevertheless suggests that IRS is a highly cost-effective addition to ITN in this endemic region.

1338

USE OF DEEP SEQUENCING FOR ASSOCIATION MAPPING OF GENES POTENTIALLY INVOLVED IN PYRETHROID RESISTANCE IN *AEDES AEGYPTI*

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Identification of target site based-resistance to insecticides has relied on detection of the single target sites mutations known to affect insecticide resistance in the field. With the advent of next generation sequencing we are now able to sample the whole genome association to detect single-nucleotide polymorphisms (SNPs) associated with insecticide resistance. This study seeks to identify SNPs associated with pyrethroid survival in natural populations of *Aedes aegypti* collected in the Viva Caucel and Vergel populations from Yucatan, Mexico. Four library sequences were built from the DNA of 25 mosquitoes. Two replicate libraries contained DNA from mosquitoes that had survived one hour exposure to a predetermined LC_{50} (25 µg a.i./bottle) and two contained DNA of mosquitoes that died from the same exposure. Sequences were obtained from an Illumina HiSeq2000/2500 Sequencer. Alignments of paired read data were run in the NextGene software, interrogating each library sequence with an insecticide resistance library of reference containing 307 genes with 4,039,599 nucleotides. SNPs with coverages <25 or >1000 were excluded as were SNPs that didn't occur in all four libraries. Log Likelihood Ratio Tests were then used to identify SNPs associated with resistance. Novel as well previously identified genes were found to be associated with resistance. Additional libraries are being sequenced to test for associations with deltamethrin exposure, and permethrin exposure in other *Ae. aegypti* field populations.

1339

INTEGRATED ENTOMOLOGICAL SURVEILLANCE IN ZAMBIA: IMPLEMENTATION OF A PHASED PROGRAM FOR DISTRICT BASED DELIVERY THROUGH ENVIRONMENTAL HEALTH TECHNICIANS

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Zambia has witnessed a rapid expansion in delivery of insecticidal based interventions such as Indoor Residual Spraying and Long Lasting Insecticidal Nets. Despite intensification of vector control programming, entomological surveillance is conducted sporadically and is geographically limited in coverage. Until now, there has been no routine, decentralized government entrenched longitudinal surveillance system that monitors localized species prevalence and supports routine processing of specimens to measure entomological impact of vector control interventions. A conceptual framework based on phased delivery of individual components of an integrated entomological surveillance system has been designed with supporting tools for districts with ongoing vector control activities. Individual components of the program support training of new and existing recruits, utilization of a standardized field surveillance protocol, data management, intra- and inter-district program performance, species composition mapping, and vector bionomics output associated with local malaria transmission. Nine Environmental Health Technicians who were trained for this program were selected to self-manage surveillance sessions in their respective sentinel sites with Community Health Worker assistance; based on their assessed training performance and their national representation. All sentinel sites were proficient in adopting a standardized collection protocol over multiple months during the wet-season and in yielding specimen data for building localized spatial and temporal species maps and associated bionomics. Findings highlight that the decentralized model of entomological surveillance is an achievable goal for national programs. Further exploration is required to address options that allow for nationally sustainable routes to multiply sentinel sites to ensure comprehensive spatial and temporal mapping of vector species and related parameters and assist the National Malaria Control Centre with evidenced based intervention selection and targeting.

1340

IS INSECTICIDE-TREATED MATERIAL (ITM) USEFUL FOR DENGUE CONTROL? PERSPECTIVES FROM RANDOMIZED CONTROL TRIALS WITH TREATED CURTAINS AND SCHOOL UNIFORMS

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Dengue is currently becoming a global public health problem. So far, vector control is the only method used to reduce dengue incidence. Our study aims at finding an alternative solution for dengue control by using insecticide-treated materials (ITM); i.e., treated curtains and school uniforms. Two randomized controlled trials were conducted separately in: 1) an urban city where 2,037 households were used to test ITM curtains, and 2) ten schools with 1,825 enrolled students used to test ITM school uniforms. Evaluation was carried out by entomological parameters and questionnaire interview of participants. The mosquito age was determined by using mosquito population age prediction method which is a tool to determine gene expression of age-related genes. The movement of *Aedes* vectors was evaluated by using sticky mosquito traps. Our results showed a significant reduction in *Aedes* density for both households (p=0.006) and schools (p=0.033) following an implementation. However, for the school trial, an average number of *Aedes* vectors increased after one month due to reduced efficacy of impregnated uniforms after frequent washing. Interestingly, the trial using impregnated curtains showed a trend of declining mean age of *Aedes aegypti*, i.e., 1.2 vs 8.6 days (p=0.0002) in treatment and control areas respectively; and a trend of increased movement of vector populations out of households after a one-year trial, i.e., 11.5% difference in treatment areas while no change was observed in control areas. Surveys with participants showed promising acceptability to the technology. In conclusion, application of ITM in dengue control was useful in either reducing dengue vectors and/or reducing their mean ages, which could have an impact on dengue transmission. We observed a reduction of dengue cases in treatment areas when compared to control areas. Further investigation is needed to decide whether an innovative control method using ITM is practical and effective for a long-term and large-scale implementation.

1341

ASSESSMENT OF THE INSECTICIDE RESISTANCE STATUS OF *AEDES AEGYPTI* IN LIMA, PERU

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In Peru, *Aedes aegypti* was successfully eradicated in 1958 after a 13-year DDT spraying campaign conducted by the Ministry of Health (MoH). However, this mosquito re-infested Lima in 2000, and dengue outbreaks were reported five years later. Current MoH practices for *Ae. aegypti* control consist of temephos applications for larval control and pyrethroid spraying for adult control during dengue outbreaks. Based on the chemical spraying history, evaluating the insecticide susceptibility of *Ae. aegypti* populations from Lima could help prevent potential chemical control failures and guide decisions for an effective mosquito vector control program. Therefore, the objective of this study was to determine the insecticide resistance status of *Ae. aegypti* from northern Lima. Centers for Disease Control (CDC) bottle bioassays were performed using 3-5 d-old F1adults. Insecticide susceptibility was evaluated following CDC diagnostic dose and time for alphacypermethrin, deltamethrin, cypermethrin, and lambdacyhalotrin (10 µg/30 min); permethrin (15 µg/30 min); fenitrothion and malathion (50 µg/30 min); DDT (75 µg/45 min), and benthiocarb (12.5 µg/30 min). *Ae. aegypti* New Orleans and Rockefeller strains were also evaluated and used as reference for insecticide susceptibility. *Ae. aegypti* F1 population from Lima was 100% susceptible to all five pyrethroids and to malathion but resistant to DDT (10%). This population was apparently less susceptible to fenitrothion (78%) and benthiocarb (3%); however, *Ae. aegypti* susceptible strains were also less susceptible to fenitrothion (<14%) and benthiocarb (<2%). Our results suggest that no insecticide resistance exists to the five pyrethroids examined and to malathion, yet this *Ae. aegypti* population is resistant to DDT. The response to fenitrothion and benthiocarb should be re-examined at different diagnostic doses and times to determine if *Ae. aegypti* from Lima are actually resistant to these chemical classes.

1342

THE PLASTICITY AND HERITABILITY OF SPATIAL REPELLENCY RESPONSES TO TRANSFLUTHRIN IN *AEDES AEGYPTI*

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The potential for spatial repellents to contribute to novel vector control approaches, especially in transmission settings unaffected by traditional tools such as indoor residual spraying and insecticide treated nets, is widely recognized as a research priority. The process of developing new spatial repellent products and strategies has been hampered, however, by the fact that work in this area involves complex behaviors that remain not well defined and/or poorly understood. *In vitro* bio-assays consistently show that disease vectors exhibit a wide range of behavioral responses to repellent chemicals in controlled experimental settings. In order to gain a better understanding of how behavior modification can impact vector populations, the plasticity and heritability of spatial repellency responses in *Aedes aegypti* following exposure to transfluthrin was investigated using a previously described high-throughput bioassay. In general, recently colonized (F_1 generation), non-mated mosquitoes were introduced into an assay system containing a chemical gradient established by dual-ended exposure chambers: a repellent chamber treated with 1.35 mg/m^3 transfluthrin and an untreated control chamber. Mosquitoes that were repelled (moving away from the treated chamber) were considered responders and labeled SRA+, while mosquitoes that were not repelled (remained inactive) were considered non-responders and labeled SRA-. After each evaluation, specimens were collected alive and segregated based on observed behavioral phenotype. We present results on 1) the reproducibility of the behaviors in individual mosquitoes retested after a 48 hour resting period and 2) the heritability of spatial repellent behavior through six generations in which male responders were selectively bred with female responders, and non-responders with non-responders.

1343

CARBAMATE AND ORGANOPHOSPHATE RESISTANCE IN *ANOPHELES GAMBIAE* ACROSS SOUTHERN GHANA: PATTERNS AND PREDICTION

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Malaria is hyperendemic in Ghana and is a major cause of death and poverty. With strong DDT resistance entrenched throughout much of West Africa, carbamates and organophosphates (OPs) are the preferred alternatives to pyrethroids for IRS. However, resistance to both insecticide

classes has been documented in *Anopheles gambiae* in West Africa: to maintain insecticide efficacy, it is important to predict how and where resistance is likely to occur and spread. *Anopheles* larvae were sampled from 18 sites spanning five distinct ecological zones in southern Ghana from March to Mid-August 2011. Adult mosquitoes were bioassayed with bendiocarb and fenitrothion. Species and molecular characterization were performed using Scott and SINE PCRs respectively. Taqman qPCR assays were used to genotype the ACE-1 G119S resistance-associated locus and ACE-1 alleles were cloned and sequencing to determine possible copy number variation. A higher level of resistance was observed to bendiocarb than fenitrothion, though phenotypes correlated across populations. M-form and S-form were found in sympatry in 15 sample sites but in varying proportions, with three sites harbouring only M-forms. ACE-1 resistant allele (119S) frequency was much higher in S than M forms and a population from Ashiaman, a rice-growing area in Greater Accra, exhibited the highest 119S frequency reported to date (68%). ACE-1 frequency was found to be the strongest independent predictor of phenotypic resistance to both insecticides. However, duplication of ACE-1 was detected, with some individuals displaying multiple distinct alleles. Further work is now required to determine the distribution and resistance-association of ACE-1 duplications in southern Ghana.

1344

MULTIPLE INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE S.L.* ACCORDING TO COTTON CULTIVATION SCHEMES IN BURKINA FASO, WEST AFRICA

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In absence of effective vaccine, the most realistic strategy to control malaria is based on vector control relied on the use of synthetic insecticides. Unfortunately, due to the emergence of insecticide resistance in natural vector populations, many malaria vector control programmes are challenging field control failures. Here, we present new data from Burkina Faso, where longitudinal and cross-sectional surveys were conducted to monitor the frequency of the L1014F kdr and G119S ace-1R mutations and the role of metabolic-based detoxifying mechanisms in contributing to insecticide resistance in field *Anopheles gambiae* populations. In Burkina Faso since 2008 two innovative cotton growth systems based on transgenic (Bt) and biological cotton were introduced using no or less insecticide for pest control prospects. In such context, a country-wide survey associating bioassays and molecular investigations carried out from 2008 to 2010 through 26 localities in Burkina Faso. Populations of *An. gambiae* tested showed during these three years survey a generalising resistance status to PYs (permethrin and deltamethrin) and decreased mortality to bendiocarb whereas they remained susceptible to OP (chlorpyriphos methyl and fenitrothion) irrespective to area. The frequency of the L1014F kdr mutation was highest in the sudan region ranging from 0.75 to 0.99 and relatively moderated in the sudano-sahelian area. Results showed also over-expression of detoxifying enzymes such as GST, oxygenases, cytochrome P450 in *An. gambiae s.s.* from the old cotton belt together with kdr and ace-1R mutations indicating the existence of multi-resistance in Burkina Faso. The geographical distribution of resistance in *An. gambiae s.l.* populations was found in sites of cotton cultivation that has expanded dramatically in the last ten years. Until the discovery of new insecticides or formulations of existing insecticides, it is crucial to integrate the regional vector resistance status in the implementation of control interventions that will preserve a long term efficacy of these vector control tools.

1345

THE IMPACTS OF VECTOR CONTROL ON THE EFFECTIVE POPULATION SIZES OF MALARIA MOSQUITOES

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The battle against malaria mosquitoes in sub-Saharan Africa is being fought with two main weapons: indoor residual spraying of insecticides (IRS) and long-lasting insecticidal net (LLIN) campaigns. Although many programs have been successful in reducing malaria infection, demonstrating the impact of these programs on vector populations is typically confounded by numerous variables associated with collection methods. Without accurate ways of measuring the impacts of vector control, it is also difficult to determine the optimal frequency for insecticide spraying to keep transmission rates low. Here, we analyzed more than 2,200 samples of three important malaria vectors - *Anopheles gambiae, An. melas*, and *An. moucheti* - from seven sites in Equatorial Guinea that were collected over the course of anti-vector programs in that country (2004-2010). Taking advantage of recently developed coalescent genetic approaches, we addressed two main questions: a) what is the impact of vector control programs on effective population size? and b) how is the effective population size effected by single insecticide spray round? We demonstrate convincingly for the first time that both IRS- and LLIN-based control resulted in dramatically lowered effective population sizes (between 55%-87%) in all populations, with the exception of a single population of *An. melas*. No such reductions were observed in negative control populations. We also found that mosquito populations are dramatically reduced following IRS rounds (65-92%), but rebounded (2,818% increase) between 3-5 months after spraying, indicating that increased spray frequency is likely to greatly improve the impact of IRS on malaria transmission. Our findings are especially important to malaria control because we were able to conclusively link anti-vector interventions to genetic impacts, a linkage that has been difficult to establish in the past.

1346

QUANTITATIVE AND QUALITATIVE ANALYSIS OF GENE DUPLICATION IN INSECTICIDE-RESISTANT *ANOPHELES* MOSQUITOES FROM WEST AFRICA

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Gene duplication is thought to provide a major source of material for evolutionary innovation. In addition to well-known point mutations that cause Ace-1 target site insensitivity for organophosphate and carbamate insecticides, Ace-1 gene duplication has been found in several West African populations of the primary malaria vector *Anopheles gambiae s.s.* Initially using a PCR-RFLP protocol we recorded excess heterozygosity at the Ace-1 resistance locus (G119S) in several field populations, consistent with the presence of a duplication phenomenon, but specific data on individual specimens could not be inferred. Here, we develop and apply quantitative real-time PCR (qRT-PCR) with SYBR Green detection, alongside both analogue and digital droplet qPCR Taqman assays to investigate

the range of allele-specific Ace-1 copy number variation occurring in natural *An. gambiae* populations in West Africa. Our results reveal that: (a) Ace-1 duplication is widespread across West Africa; (b) is present in unexpectedly high copy number (at least five-fold); and (c) multi-copy resistant homozygotes are not uncommon, despite strong prior evidence for fitness costs in single-copy homozygotes. The pairing of the G119S Taqman assay with our newly-developed copy number qRT-PCR assay provides an informative paired-diagnostic to assess the consequences of Ace-1 mutation and duplication for insecticide resistance phenotypes and fitness in wild *An. gambiae* populations.

1347

RISE OF MUTATION CYS 1534 IN THE VOLTAGE GATED SODIUM CHANNEL GENE IN *AEDES AEGYPTI* IN MEXICO

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Aedes aegypti, is the primary vector to humans of dengue and yellow fever flaviviruses (DENV, YFV), and is a known vector of the chikungunya alphavirus (CV). Because vaccines are not yet available for DENV or CV or are inadequately distributed in developing countries (YFV), management of *Ae. aegypti* remains the primary option to prevent and control outbreaks of these arboviral diseases. Permethrin is one of the most widely used active ingredients in insecticides for suppression of adult *Ae. aegypti*. In 2012, we documented a replacement mutation in codon 1,534 of the voltage-gated sodium channel gene (para) of *Ae. aegypti* that encodes an cysteine rather than a phenylalanine and confers resistance to permethrin. A total of 86 field collections containing 4,014 *Ae. aegypti* were made throughout México from 1999 to 2012. These mosquitoes were analyzed for the frequency of the Cys1,534 mutation using a melting-curve PCR assay. Dramatic increases in frequencies of Cys1,534 were recorded from the late 1990's to 2012 in several states including Nuevo Leon in the north, Veracruz on the central Atlantic coast, and Yucatan, Quintana Roo and Chiapas in the south. From 1999 to 2012, the overall frequency of Cys1016 was 0.28. In 2000 in Veracruz the frequency was very low and by 2012 the frequency rose to 0.93. In 2008 Martinez de la Torre and Coatzacoalcos had frequencies of 0.94-1. In 2012 the frequency increased to 0.97 and had become fixed in Tuxpan. The earliest detection of Cys1,534 was in Chiapas, Guerrero and Veracruz in 2000. In total, we document a dramatic increase in the frequency of the Cys1,534 mutation in Mexico from 1999 to 2012. This may be related to previous extensive use of DDT and continued heavy use of permethrin. A rotational schedule utilizing different classes of adulticides should be implemented to slow or prevent fixation of Cys1534.

1348

BITING BEHAVIOR AND HIGH RESOLUTION MELTING DETECTION OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* IN MALI

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Understanding the behavior and detecting insecticide resistance in malaria vectors have important implications for malaria control. In 2012-2013, in four traversal passages (start, middle and end of transmission, and dry season), we have collected mosquitoes in two rural areas, Dangassa and Dioro in Mali, using human landing catches. We have used WHO bioassays to detect phenotypic resistance, and high resolution melting (HRM) technology to detect target-site mutation frequencies in the kdr locus. Preliminary results reveal a nearly even split in the proportion of mosquitoes biting indoors vs. outdoors. The proportions were 47.7% vs. 54.3% (n=1013) in July 2012, 55.7% vs. 44.3% (n=517) in October 2012, 49.7% vs. 50.3% (n=155) in December 2012 and 38.0% vs. 62.0% (n=21) in April 2013 in Dangassa. In Dioro, they were 42.8% vs. 57.2% (n=35) in July 2012, 49.7% vs. 50.3% (n=151) in October 2012, 49.7% vs. 50.3% (n=155) in December 2012 and 57.1% vs. 42.9% (n=7) in April 2013. WHO susceptibility assays detected substantial resistance to DDT at both sites (22% and 14% mortality rates in Dangassa and Dioro, respectively) and susceptibility to bendiocarb and pirimiphos-methyl. The HRM analysis for kdr genotypes conducted on a subsample showed frequencies of 0.2 (RR), 0.3 (RS) and 0.5 (SS) at the start of the rainy season, and 0.2 (RR), 0.0 (RS) and 0.8 (SS) during the middle of the rainy season in Dangassa. In Dioro, the kdr allele frequencies were 0.4 (RR), 0.3 (RS) and 0.4 (SS) at the start of the rainy season and 0.4 (RR), 0.2(RS) and 0.4 (SS) during the middle of the rainy season.

1349

CHARACTERIZATION OF INSECTICIDES RESISTANCE IN *AEDES AEGYPTI* POPULATION FROM THE CARIBBEAN REGION OF COLOMBIA

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We determined the susceptibility to insecticides and biochemical and molecular mechanisms involved in insecticide resistance of nine populations of *Aedes aegypti* in the Caribbean Region of Colombia. Bioassays were performed for temephos in larvae according to WHO and bottle bioassays for adults with the insecticides: lambdacyalothrin, cyfluthrin, permethrin, deltamethrin, malathion, fenitrothion and pirimiphos-methyl The resistance ratios were calculated using the susceptible Rockefeller strain as a control. Additionally, the organochloride DDT was evaluated through the impregnated papers technique. Biochemical resistance mechanism were identified associated with high level of α, β-esterases, mixed-function oxidases, insensitive acetylcholinesterase and glutathione S-transferases; we identified the mutation Ile1,016 in the gene of the voltage-dependent sodium channel and its frequency. All populations were susceptible to the organophosphates evaluated (RR=1x-4x) with exception of Puerto Colombia and Soledad (Atlántico) strains which demonstrated high and moderate resistance to temephos (RR=15x) and (RR=5x), respectively and Sincelejo (Sucre) with moderate resistance to pirimiphos-methyl (RR=5x). All populations were resistant to DDT (2-28% mortality). Strains evaluated exhibited values of resistance to lambda-cyalothrin between 4,9-83 fold, for deltamethrin between 0,9-37,8 fold, cyfluthrin with 0,5-33,8 fold and permethrin of 1,8 -17,9 fold. Over-expression of glutathione S-transferases were found in all populations with the exception of Puerto Colombia (Atlántico) and Cartagena (Bolivar); as well as α-esterase in strains: Valledupar (Cesar) and Monteria (Cordoba); and insensitive acetylcholinesterase in Puerto Colombia strain (Atlantico). The mutation Ile1,016 was registered in all populations with variability in its frequency.

1350

HYDROLOGICAL DISTURBANCE AFFECTS COMPETITION BETWEEN *AEDES* VECTOR MOSQUITOES VIA CHANGES IN LEAF LITTER

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The invasive mosquito *Aedes albopictus* utilizes water-holding containers for its development where it competes for food as larvae with the native *Aedes triseriatus* in the eastern United States. We tested the hypothesis that prior hydrological disturbance would affect competition between *Ae. albopictus* and *Ae. triseriatus* in containers via changes in leaf litter decomposition, associated microbial resources, and leached tannins. Containers provisioned with senesced litter were treated to mimic three broad hydrological regimes experienced by containers in nature: dry, flooded, and a wet-dry cycle, before varying densities of competing first-instar *Ae. albopictus* and *Ae. triseriatus* larvae were added using a response surface design. We found that hydrological regime affected litter resource quality, water quality, and *Aedes* competition. Previously dry leaf litter decayed more slowly, supported lower microbial abundance, and leached higher tannin concentrations than litter that had been flooded or exposed to a wet-dry cycle. Containers with previously dry litter experienced more intense competitive effects of *Ae. albopictus* on *Ae. triseriatus* population performance than containers that had previously been flooded or exposed to a wet-dry cycle. In contrast, prior hydrological regime did not affect the population performance of *Ae. albopictus.* These results suggest that prolonged wetter conditions prior to *Aedes* utilization of container habitats may relax competitive effects of *A. albopictus* on *A. triseriatus,* and help foster coexistence between the two species. Coexistence of these *Aedes* mosquitoes has implications for understanding mosquito invasions generally and specific disease risks in eastern North America.

1351

DESIGN AND TESTING OF A NOVEL, PROTECTIVE HUMAN-BAITED TENT TRAP FOR THE COLLECTION OF ANTHROPOPHILIC DISEASE VECTORS

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Currently, there exists a deficit of safe, active trapping methods for the collection of host-seeking *Anopheles* and other disease-causing arthropod vectors. The gold standard approach for mosquito collection is that of Human Landing Catch (HLC) in which an individual exposes bare skin to possibly infected vectors. Here, we present the development of a new method for mosquito collection, the InfoScitex Tent (IST), which utilizes modern tent materials coupled with a novel trap design. This provides an efficacious, non-labor intensive, and safe method for vector collection. In these initial studies, we found it collected an average of 31.5 *Anopheles gambiae s.l.* per trap per night in rural villages in Southeastern Senegal, and 42.5 *Culex* group V per trap per night in the semi-urban town of Kedougou, Senegal. In direct comparisons to HLC, the tent was not statistically different for collection of *Cx. quinquefasciatus* in crepuscular sampling, but was significantly less efficacious at trapping the highly motile dusk biter *Aedes aegypti*. These studies suggest that the IST tent is a viable and safe alternative to HLC for *Anopheles* and *Culex* sampling in areas of high vector-borne disease infection risk.

1352

HETEROGENEITY IN MALARIA VECTOR DYNAMICS AND BIONOMICS IN NCHELENGE DISTRICT, ZAMBIA

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As part of the Southern Africa International Centers for Excellence in Malaria Research (ICEMR) project, mosquito collections were performed from March-May 2013 in Nchelenge District, Luapula Province, Zambia. Located along the environs of Lake Mweru and Kenani Stream, Nchelenge experiences hyperendemic transmission and has the highest malaria infection rate in children under the age of 5 years despite implementation of indoor residual spraying (IRS) and long-lasting insecticide-treated net (LLIN) distribution. Center for Disease Control light traps (CDC LTs), pyrethroid spray catch (PSC), and larval collections were performed at three villages along Lake Mweru and two villages along Kenani Stream. The collections revealed that *Anopheles gambiae* sensu stricto is the dominant vector in the lakeside villages, whereas An. *funestus s.s.* is the primary vector with secondary contribution from *An. gambiae s.s.* in the streamside villages. Both human malaria infection rates and vector populations were higher near the streamside villages than those of the lakeside villages. Surveys of potential oviposition sites found that temporary water bodies near the stream and the stream itself are the major breeding sites for *An. gambiae* and An. *funestus*. Both vector species are highly anthropophilic and are predicted to have high sporozoite infection rates. The differences in human malaria infection rates and mosquito abundances between the lake and stream sites support the hypothesis that heterogeneity exists in the human blood index, entomological inoculation rates, and multiple blood feeding behavior of the two vectors within Nchelenge District. The insecticide resistance status of both malaria vectors will also be explored. The vector data in Nchelenge present unique opportunities to further our understanding of malaria transmission and the implications for malaria control in high-risk areas.

1353

CEMETERIES ARE EFFECTIVE SITES FOR SURVEILLANCE OF LA CROSSE VIRUS AND VECTOR POPULATIONS IN APPALACHIA

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In North America, the mosquito-borne disease La Crosse encephalitis is the leading cause of arboviral disease among children, and was previously limited to the upper Midwest. Unfortunately, the Appalachian region, with Tennessee in particular, now has the highest incidence risk in the nation: 228.7 cases per 100,000 children 15 years and younger, and almost 75% of all US cases reported in a year are in Appalachia (Haddow and Odai 2009). In 2012, nine pediatric cases of La Crosse encephalitis occurred in eastern Tennessee, including one death. While *Aedes* (*Ochlerotatus*) *triseriatus* has been the historical vector, *Ae. albopictus* and *Ae. (Oc.) japonicus* are two invasive species that may be important accessory vectors. All three vectors oviposit desiccant-tolerant eggs in forest stands and opportunistically oviposit eggs in artificial containers. Use of artificial containers may move La Crosse virus (LACv) from the forest's tree holes and into the urban environment as LACv can be transmitted to mosquito offspring (transovarial transmission). In an attempt to detect LACv in active mosquito populations, our objective was to determine if cemeteries were effective sites for monitoring LACv and the vector population; consequently, we conducted an in-depth vector ecology study centered around the 2012 fatal case. Briefly, 38 cemeteries were selected within 10 radial miles of the fatal case. At each cemetery, four ovitraps baited with water and seed germination paper (egg paper) were placed at the four cardinal directions. Egg papers and water were replaced weekly, from 5

Sept. - 3 Oct. 2012, this yielded a total of 760 egg papers. Recovered egg papers (99.3%) were brought back to the laboratory where eggs hatched and adults emerged. Thus far, we have successfully recovered all 3 vector species representing *Ae. (Oc.) triseriatus* (87.6%), *Ae. albopictus* (12.2%) and *Ae. (Oc.) japonicus* (0.2%), and identified four positive pools of *Ae. (Oc.) triseriatus*. This preliminary data indicates cemeteries are effective sites for surveillance of LACv and vector populations.

1354

ENVIRONMENTAL INVESTIGATION FOLLOWING A LA CROSSE ENCEPHALITIS CASE FATALITY IN TENNESSEE, 2012

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La Crosse encephalitis virus (LACV) is an important cause of pediatric encephalitis in the United States. Historically, human cases have been concentrated in the upper-Midwestern states, but in the mid-1990s , the Appalachian region including east Tennessee became a focal point. In 2012, nine pediatric cases of LACV encephalitis occurred in Tennessee, including one death. To detect LACV in the area, oviposition traps, BG sentinel and CDC light traps were placed at forty-nine sites consisting of cemeteries and houses within ten miles of two pediatric infections including the deceased child from September 5 to October 3, 2012. Ninety-one papers have had adults reared and pooled so far. The pools were tested for LACV by real-time RT PCR. Adult collections from BG and CDC traps at house sites were comprised of 36% *Aedes albopictus,* 29% *Culex erraticus*, 13% *Anopheles punctipennis*, 9% *Cx. pipiens*, 8% *Ochlerotatus triseriatus*, 2% *Ae. vexans*, 2% *An. quadrimaculatus* and 1% other species. Adults emerging from cemetery collected egg papers were *Oc. triseriatus* (87.6%), *Ae. albopictus* (12.2%) and *Oc. japonicus* (0.2%). Papers collected from house sites showed *Oc. triseriatus* (54.8%), *Ae. albopictus* (44.6%) and *Oc. japonicus* (0.6%). During the last two weeks, the percentage of *Oc. triseriatus* emerging from the papers decreased; whereas *Ae. albopictus* increased for house and cemetery sites. Of house sites, 50% showed a composition of two species, 37.5% with three and 12.5% with one. Of cemetery sites, 57.7% had a species composition of two, 27% with one and 11.5% with three species. Some (3.8%) cemetery sites did not hatch. To date, 628 pools of mosquitoes have been tested. All 39 pools from BG and CDC trap collections were negative for LACV. Four pools of *Oc. triseriatus* from egg paper collections were LACV positive. The positive pools came from a cemetery site on October 3, 2012. Along with the successful detection of LACV, these findings suggest a temporal and spatial variation in mosquito activity. *Aedes albopictus* may also be more prevalent near homes and *Oc. triseriatus* near cemeteries.

1355

FIELD EVALUATION OF A PUSH PULL STRATEGY TO CONTROL MALARIA VECTORS IN NORTHERN BELIZE, CENTRAL AMERICA

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Current vector control tools are quickly becoming inadequate for controlling arthropod-borne diseases such as malaria. The reasons for this are complex but combined highlight the need for development of novel approaches to reduce pathogen transmission. Efforts are being carried out to evaluate the use of spatial repellents and mosquito traps in a combined push-pull strategy to reduce the probability of human-vector contact in and around homes. Here, we report on a 16-night, four-arm Latin square experimental hut study in Belize, Central America that evaluated the ability of this approach to reduce densities of two locally relevant malaria vectors, *Anopheles vestitipennis* and *An. albimanus*, from entering the structures . Utilizing a matched-control (untreated) hut, we measured changes in vector entry patterns at huts receiving either indoor repellent alone (1.4 mg/m^3 transfluthrin), outdoor traps (CDC miniature light traps baited with human foot emanations), or both interventions simultaneously. Outdoor light trap yields were also compared between huts with and without repellent. Results show that while light traps alone did not impact mosquito entry into huts, use of repellent alone significantly reduced mosquito entry by more than 60% (± 4%) for both species. The combined intervention did not result in any further reduction of mosquito entry over repellent alone. In fact, while not significant in terms of absolute numbers of mosquitoes entering the huts, a post-hoc Wilcoxon Signed Rank analysis indicates that the presence of a baited CDC light trap outside of a hut may reduce the repellency effect of transfluthrin. Interestingly, use of an indoor repellent did increase the average numbers of *An. vestitipennis* (an endophagic species) captured in outdoor light traps by 50% (±27%), but no corresponding effect was seen with *An. albimanus* (an exophagic vector). These results indicate that while a combined push-pull intervention has the potential to reduce human-vector interactions, the baseline ecology and behaviors of the target vector(s) will influence efficacy.

1356

INSECT-SPECIFIC VIRUSES DETECTED IN LABORATORY MOSQUITO COLONIES: IMPLICATIONS FOR EVALUATING VECTOR COMPETENCE EXPERIMENTS

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In the past 5 years, there has been a dramatic increase in the detection and description of insect-specific viruses found in field-collected mosquitoes. Evidence suggests that these viruses are widespread in nature and many appear to be maintained by vertical transmission (infected female transmits virus to her progeny). Recent studies also indicate that superinfection exclusion (cells infected with one virus are refractory to infection by a second related virus) may occur between some insect-specific viruses with pathogenic arboviruses, thus altering the vector competence profiles of certain mosquito species. In order to evaluate this phenomenon further, we initiated studies to investigate the presence of insect-specific viruses in our laboratory mosquito colonies. Pools containing 50 male and 50 female mosquitoes collected from each colony were homogenized and virus isolation was attempted in Vero (vertebrate) and C6/36 (invertebrate) cell lines. Cell cultures were examined for cytopathic effect and also screened by electron microscopy for the presence of virus-like particles. Total RNA was extracted from C6/36 cell cultures and submitted for deep sequencing with an Illumina platform. Seven out of 14 colonies were found to contain an insect-specific virus. Phylogenetic analyses and serological tests confirmed the presence of previously described insect-specific flaviviruses as well as several novel viruses. The infection rates detected within the infected mosquito colonies were variable. The potential implications of these findings in regards to vector competence studies will be discussed.

1357

THE EFFECTS OF TRANSIENT IMMUNE ACTIVATION ON TRANSGENIC *ANOPHELES STEPHENSI* FITNESS

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Mosquitoes of the genus *Anopheles* spread the *Plasmodium spp.* parasites responsible for human malaria. Rising resistance and difficulties of distribution have hampered traditional malaria control efforts, which have focused on chemotherapeutic agents to treat cases as they arise and control of mosquito populations through insecticide use, bednets and habitat removal. These issues, coupled with the lack of an effective

vaccine, call for the development of new malaria control methods. Multiple laboratory groups have created transgenic mosquitoes refractory to malaria infection, but no such lines have yet been released as part of a malaria control program. One problem with genetically modified mosquito lines is that they are generally assumed to be less fit than their wild-type conspecifics, which would stop them from replacing the native population and limit their effectiveness. However, previous studies in *Drosophila* and initial data from mosquitoes indicate that temporary immune induction in transgenic insects may have minimal effects on fitness. Therefore, we set out to investigate how short-term induction of the mosquito immune system affects mosquito fitness. We compared various aspects of mosquito fitness, such as; lifespan, fecundity, development time, mating competitiveness, wing length and blood meal consumption in five separate transgenic lines to the same measures in wild-type mosquitoes and have seen few effects. The transgenic lines tested were chosen to test different aspects of transgenesis that may affect fitness, and include the same gene under different promoters, different genes under the same promoter, different isoforms of the same gene and different insertion points of the same construct. These results suggest that the mere presence of a transgene in mosquitoes does not necessarily lead to a large fitness reduction, and indicate that genetically modified mosquitoes may soon be a viable tool for the control of vector-borne diseases.

1358

MODERATE PRECIPITATION CONDITIONS FAVORED INCREASE OF MOSQUITO POPULATION VECTORS OF VENEZUELAN EQUINE ENCEPHALITIS IN LA ALTA GUAJIRA COLOMBIA

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In the past 17 years none outbreak of Venezuelan equine encephalitis (VEE) has been registered in La Guajira and the disease is no longer a public health priority. However, it is important to consider the last two epidemics that took place in 1992 and 1995 when the virus showed that it was still a threat, after its disappearance was speculated. The objective of this work was to monitorize the precipitation conditions that favor the increase in the populations of mosquito vectors (*Diptera: Culicidae*), in order to assess the entomological risk of virus transmission. Different precipitation periods were studied from September 2009 to August 2012 in La Guajira. Mosquitoes were collected using CDC traps and identified to species. Daily average abundance of female mosquito vectors species collected per night was calculated, and compared to the accumulated precipitation register of the previous 16 days of the collecting day. Vector species, *Aedes taeniorhynchus* and *Psorophora confinnis*, achieved their maximum abundance, when rainfall was moderated between 30 to 60 mm., either heavy (above 80 mm), very low or absence rainfall affected negatively their populations. Both species proved to need specific and slightly different climatic conditions. In conclusion the entomological risk of transmission of VEE increases in the rainy season, particularly at the end of it.

1359

TEMPORAL CHANGE IN *ANOPHELES DARLINGI* DIVERSITY IN SOME MALARIA ENDEMIC PERUVIAN LOCALITIES

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WHO reported approximately 500,000 malaria cases in the Americas in 2011, of which 22,878 cases were in Peru. In the city of Iquitos in northern Amazonian Peru, *Anopheles darlingi* is the primary malaria vector. The overall aim of this project is to evaluate vector control measures and seasonality by measuring local allelic diversity (*A*) and effective population size (N_E) in *An. darlingi*. Based on earlier studies that detected mid-range N_E and high gene flow in villages surrounding Iquitos, we hypothesize that neither diversity nor N_E will vary among localities seasonally, although this has not been tested previously. A quantitative measure of vector control effectiveness is a significant reduction in N_E and *A*. *An. darlingi* microsatellite data from fifteen *loci* were analyzed for four localities south and west of Iquitos: San Jose de Lupuna (LUP), Villa El Buen Pastor (VBP), Cahuide (CAH) and Santo Tomas (STO). Genetic diversity, differentiation (F_{ST}), N_E, departures from Hardy-Weinberg equilibrium and linkage disequilibrium were measured for 17-50 specimens per collection. Sequential Bonferroni corrections minimized multiple testing biases. Both structure and F_{ST} analyses detected one population of *An. darlingi*, similar to findings of Mirabello et al. (2008). However, in the current study, N_E estimates were lower. Overall genetic diversity was high and similar for the four localities. These results differ from those of Pinedo-Cancino et al. (2006) who used amplified fragment length polymorphism analyses and reported limited diversity in *An. darlingi* in the same region. Differences between February (dry) and April (rainy) in 2011 were only assessed for LUP and VBP. In this analysis, mean *A* for all *loci* was stable in both localities, but in VBP, observed heterozygosity (H_O) decreased and expected heterozygosity (H_E) increased. Our results suggest local seasonal environmental changes may influence diversity within this population of *An. darlingi*.

1360

A PHARMACOLOGICAL APPROACH TO VECTOR CONTROL VIA THE *ANOPHELES GAMBIAE* SEX PEPTIDE RECEPTOR

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While many vector-targeted control strategies aim to decrease vector survival, there are many mosquito behavioral processes that could serve as targets for strategies that would decrease vectorial capacity. Among such behaviors, mating is a potential target for intervention in many insects, including *Anopheles gambiae*, because there is a dramatic increase in female refractoriness to mating after a single initial mating event. In other insects, including D. melanogaster, sex peptide receptor (SPR) has been shown to play a significant role in regulating mating behavior. SPR is a G protein-coupled receptor that is activated by sex peptide (SP), which is present in the male seminal fluid, and by myoinhibiting peptides (MIPs), which are thought to be ancestral ligands for SPR-related receptors. We are investigating the pharmacology of SPR in *An. gambiae* by screening selected MIPs in cell-based assays to identify receptor agonists. Using an agonist-based approach, it may be possible to induce female refractoriness to mating by delivery of these peptides *in vivo*, and thereby decrease

reproductive ability and fitness of agonist-treated mosquitoes, leading to source reduction. We have found that RNAi-based knockdown of the D. melanogaster SPR ortholog leads to pre-adult developmental arrest and impaired flight ability, suggesting that SPR antagonists may be of interest for control of *An. gambiae* if the mosquito receptor plays similar roles. The goals of this project are to better understand SPR receptor-ligand activity relationships and to investigate the role of SPR in mosquito behavior and survival, in an effort to validate novel drug targets for development of next-generation insecticides.

1361

FUNCTIONAL CONFIGURATION OF METAGENOME IN THE MOSQUITO GUT ECOSYSTEM

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Host associated microbes are ubiquitous, yet our understanding of the interactive relationships is very limited. The mosquito gut ecosystem accommodates a complex microbial assemblage. The dynamic gut microbiome profoundly affects various mosquito life traits, such as fecundity and immunity. Besides, bacteria may directly interfere with malaria *Plasmodium* development in the gut before invasion occurs. However, little is known about the genetic structure and functional repertoire of the gut microbiome. In this study we generated 15Gbp metagenomic DNA- and RNA-seq data from the guts of adult mosquito *Anopheles gambiae* under conditions with sugar meals or blood meals. Using an assembly-based pipeline, a 37.1 Mbp metagenomic reference was compiled, which included 49,000 contigs. Similarity based taxonomic classification recognized at least 6 phyla, predominant taxa included Proteobacteria (Enterobacteriacea, Psedomonadaceae and Acetobacteracea) and Bacteroidetes (Flavobacteriaceae). The function annotation was implemented via SEED/Subsystems and COG/KEEG, which recognized 23,550 coding sequences. Among them 42% were assigned into ~700 subsystems. Metabolic reconstruction predicted 1658 reactions and 1266 compounds. In addition to the presence of many ABC transporters, there are large numbers of TonB dependent transporters and polysaccharide utilization *loci*, constituting uptake systems for iron, vitamin B 12 and various biopolymers. The presence of large capacity of resistance to antibiotics and toxic compounds may represent a defense strategy for maintaining community stability. The metagenomic reference was further used for mapping RNA-seq reads to decipher context dependent community functions, which was exemplified by metatranscriptomic analysis of the sugar-fed and blood-fed guts. The metagenomic reference provides insights into the taxonomic and functional configuration in the mosquito gut ecosystems.

1362

DIVERSE SYMPATRIC MALARIA VECTOR SPECIES IN PURSAT PROVINCE, WESTERN CAMBODIA, AN AREA WHERE ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* IS HIGHLY PREVALENT

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Anopheles mosquitoes from a two-year longitudinal entomological collection in Thmar Da commune, Pursat Province, were analyzed to determine which species transmit malaria to humans along Cambodia's border with Thailand. This region has been a hotspot for the evolution of drug-resistant *Plasmodium falciparum* parasites for decades, so understanding the complex transmission dynamics and the vector species responsible for spreading these parasites is critical for effective malaria prevention, control, and eventual elimination. Using human landing catch and CDC light trap methods, we collected 4,264 anophelines comprising 14 different morphologically-identified species (*An. barbirostris, An. dirus, An. hyrcanus, An. hyrcanus group, An. jamesii, An. karwari, An. kochi, An. maculatus, An. minimus, An. nigerrimus, An. philippinensis, An. tessellatus, An. umbrosus*, and *An. vagus*), all of which have been incriminated as (mostly secondary) malaria vectors elsewhere in southeast Asia. Specimens were analyzed for (i) *Plasmodium* infection using a nested PCR, (ii) bloodmeal source (i.e., human or domestic animal), and (iii) the presence of cryptic molecular species defined by rDNA ITS2 *loci*. Preliminary molecular speciation reveals even more species diversity in this area, with multiple cryptic species present. Several different anopheline species, including *An. maculatus*, *An. dirus* A, and *An. tessellatus*, were found to carry *Plasmodium* parasites. The implications of multiple vector species and their biting behaviors for malaria control and transmission in this region will be discussed. The diversity of vector species in Thmar Da and elsewhere in Cambodia is a challenge for vector control efforts and underlies the need for further characterization of vector ecology, behavior, and population genetics in this country's malaria-endemic areas.

1363

IDENTIFICATION OF *AEDES AEGYPTI* IMMUNE RESPONSES MECHANISMS TO DENGUE VIRUS

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In recent years there has been considerable progress in our knowledge of dengue, particularly in vaccine development, the characterization of the immune responses and molecular properties of the virus. However, there are still many aspects that must be investigated in terms of its transmission by the principal vector, *Aedes aegypti*. We have conducted research to elucidate Dengue virus-vector relationships, specifically the innate immune response of *A. aegypti* to dengue virus infection. For this, we identified and selected two strains of *A. aegypti*, from Cali, Colombia with different susceptibility to dengue infection: Susceptible (Cali-S) and refractory with midgut infection barrier (Cali-MIB). We compared the global gene expression of the midguts of Cali-S and Cali-MIB after ingestion of sugar, a bloodmeal, or a bloodmeal containing Dengue-2 virus using microarrays. Preliminary results from the microarrays indicated the expression of a total of 3761 genes. Of these, a total of 165 immune-related genes have been identified. A differential expression between the two strains exposed to DENv-2 virus included genes in different functional groups; immunity, metabolism, proteolysis, redox, replication, transport, and unknown function. Characterization of these genes is underway to elucidate if refractoriness is related to an upregulation or downregulation of specific or multiple genes in the two strains. This study will provide a global overview of gene expression in susceptible and refractory mosquitoes and will be compared with other studies that have looked at specific molecules and pathways. This study will also validate the use of our field derived strains as an important biological model to study Dengue-vector relationships.

1364

OCCURRENCE OF NATURAL *ANOPHELES ARABIENSIS* SWARMS IN AN URBAN AREA OF BOBO-DIOULASSO CITY, BURKINA FASO, WEST AFRICA

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The swarming behavior of natural populations of *Anopheles arabiensis* was investigated by conducting transect surveys on 10 consecutive days, around dusk, from March to April and from September to October 2012 in Dioulassoba, a district of Bobo-Dioulasso city in Burkina Faso (West Africa). Swarms were observed outside, around identified larval breeding sites on the banks of the Houet River, as well as in the open-air courtyards found at the centre of many homes in the region. Swarms were found to occur in open sunlit spaces, mostly located above physical or visual cues somehow visually distinct from the surrounding area. Overall 67 and 78 swarms were observed, respectively, during the dry season (March-April) and the rainy season (September-October) of 2012, between 1.5 and 4.5 meters above the ground at their centre. 964 mosquitoes were collected and analyzed from dry season swarms, of which most were male, and all were *An. arabiensis*, as were the few resting mosquitoes collected indoors. Larvae collected from breeding sites found on the banks of the Houet River mostly consisted of *An. arabiensis* and only a minority *An. coluzzii* (formerly identified as *An. gambiae* M form). Of 1694 mosquitoes analyzed from 78 swarms in the wet season collections, a few *An. gambiae* males were identified, and the remainder was *An. arabiensis*. The majority of larvae collected during the wet season from the same breeding sites were identified as *An. arabiensis* and only a minority *An. coluzzii* form and even fewer *An. gambiae* (formerly known as *An. gambiae* S form). The same pattern of species composition was seen in resting mosquitoes, though the proportion of *An. arabiensis* was less overwhelming. These data support the conclusion that *An. arabiensis* is the most prevalent species in this area, though the difference in species composition when using different population sampling techniques is noteworthy. Further studies are required for more detailed investigation of male dispersal, feeding behaviour and mating patterns in an urban setting.

1365

ACCURATE SPECIES IDENTIFICATION IS CRITICAL FOR MALARIA CONTROL: THE UTILITY OF MOLECULAR CHARACTERIZATION OF ANOPHELINE SPECIES ACROSS INDONESIA, A COUNTRY OF DIVERSE VECTORS AND MALARIA TRANSMISSION

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Identification of malaria vectors is critically important for the evaluation of malaria transmission dynamics. In areas of high biological diversity, morphological species identification may not fully describe the amount of variation that is relevant to malaria transmission. The burden of malaria in Indonesia, a region of high biological and geographical diversity, is significant and varies across the archipelago, largely due to differences in the types of mosquito species inhabiting each region. In many areas in Indonesia, there are multiple sympatric anopheline species whose specific bionomic traits ultimately determine the dynamics of malaria transmission. Most of these species are isomorphic members of cryptic species complexes. In this study, we used molecular tools to identify anopheline specimens collected from four different sites in Indonesia to molecular species to address site-specific species identification issues as they relate to malaria control. Specimens were collected from four field sites in Indonesia: a low transmission field site in Purworejo, Central Java; a medium transmission field site in Lampung, Sumatra; and high transmission sites in South Halmahera and Papua. 2,840 anopheline samples from different entomological collections, representing 18 different morphological species, were sequenced for ribosomal DNA ITS2 using Sanger sequencing. Molecular species identification revealed 22 different molecular species, a high level of misidentification, and 9 species carrying *Plasmodium falciparum* or *P. vivax* sporozoites. These species include: *Anopheles aconitus, An. balabacensis, An. farauti 4, An. indefinitus, An. kochi, An. maculatus, An. sundaicus A, An. vagus,* and *An. vanus*. Accurate species identification is cost-effective for control programs and site-specific evaluation of species compositions at the molecular level is recommended prior to the implementation of any control or monitoring program. These results will contribute to our understanding of the distribution of vector species, their behavioral patterns, as well as provide new diagnostic tools.

1366

PRESENCE OF *AEDES (STEGOMYIA) ALBOPICTUS* (SKUSE,1894) (*DIPTERA: CULICIDAE*) IN COLOMBIA

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Aedes (Stegomyia) albopictus (Skuse, 1894), it is the dengue´s vector, yellow fever in Southeast Asia and others arbovirus such as chikungunya fever; this vector is an invasive specie that has the ability to reproduce in natural and artificial environments with a widely geographic distribution, being in different countries in Europe, Africa and America. The first record of *Ae. (Stg.) albopictus* in South America was at Brazil in 1986, followed by Bolivia, Colombia, Paraguay, Argentina, Uruguay and Venezuela. Also, inside of the entomological surveillance that is made in Colombia to exotic species of public health importance, we includes the sentinel surveillance sampling is performed in larvitraps and in some cases ovitraps at strategic points such as airports, land and river ports. Being the first record of *Ae. (Stg.) albopictus* in Leticia – Amazonas (Colombia) in 1998, this place is the border zone with Tabatinga-Brazil and Caballo Cocha, Iceland and Santa Rosa - Perú; after that we found this new vector in six of the thirty-two Colombian departments, starting with the Special District, Industrial, Port, and Ecotourism Biodiversity Buenaventura-Valle del Cauca, 2001. Although there are few records of its role as a vector in the Americas, there is one report of natural infection in *Ae. (Stg.) albopictus* with serotypes Den-1 and Den-2 in Colombia in 2006, specimens from the municipality of Buenaventura, Valle del Cauca. Therefore it is likely that this mosquito in the future become a efficient vector of dengue and other arboviruses in our country continue to be important sentinel surveillance through larvitraps and ovitraps and integrate the control of *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* in the country, as globalization has enabled the generation of new trade routes and through passive transport in less time get more places both the mosquito vector and the virus and the sick.

1367

TUBERCULOSIS IN LAMBARÉNÉ, GABON: FIRST EPIDEMIOLOGICAL AND MICROBIOLOGICAL DATA

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The central African region is highly affected by the pandemics of HIV and tuberculosis (TB), but systematic data on local epidemiology and drug resistance are scarce, if ever available. The objective of this first prospective observational cohort analysis of 200 TB patients in Lambaréné, Gabon, is to describe demographic, clinical and microbiological characteristics and evaluate treatment outcomes. Patients from three different treatment centers in Lambaréné were included and followed up 2 and 6 months after treatment initiation. Sputum samples were sent to Germany for culture and drug sensitivity testing. To date 120 patients have been included; 74 (62%) were male and 20 (17%) were children. 105/120 (88%) were new TB cases, in 9/120 (8%) and 5/120 (4%) retreatment was started due to default and relapse, respectively. Among the adult patients 75/100 (75%) presented with smear positive pulmonary TB, 18/100 (18%) with smear negative pulmonary TB and 7/100 (7%) with extra-pulmonary TB. HIV co-infection was confirmed in 36/120 (30%), in 12/120 (10%) HIV status was unknown. Of the 54 positive sputum culture results obtained so far 47/54 (87%) were identified as *Mycobacterium tuberculosis* and 7/54 (13%) as *M. africanum*. Full drug sensitivity for the first-line antituberculous drugs (RHZES) was ascertained in 44/54 (81%) patients. Resistance to at least rifampicin and isoniazid (multi-drug resistance, MDR) was found in 3/54 (6%), and mono-resistance to isoniazid and streptomycin in 3/54 (6%) each, and combined resistance of isoniazid plus streptomycin in 1/54 (2%). So far treatment outcome could be evaluated for 36 patients; 17/36 (47%) were classified cured, 3/36 (8%) defaulter, 1/36 (3%) treatment failure, 10/36 (28%) lost to follow-up, and 5/36 (14%) deceased. All deceased patients were HIV co-infected. These first interim results indicate that in Gabon TB is a serious public health threat with a high mortality in HIV co-infected patients and a low cure rate. Besides improvement in basic TB control, implementation of mycobacterial culture and drug sensitivity testing beyond research purposes as well as the establishment of a second-line regimen are urgently needed to halt the further spread of MDR TB.

1368

IMPACT OF RESPIRATORY ILLNESSES DURING PREGNANCY ON NEWBORN'S WEIGHT - A COMMUNITY BASED LONGITUDINAL STUDY AT AN URBAN SLUM IN PAKISTAN

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Birth weight is a powerful determinant of an infant's long term growth and survival. Although maternal health is widely believed to impact the birth weight of the baby, the exact factors during pregnancy which influence the birth weight are not clearly known. We are conducting a longitudinal observational study at Bilal Colony, a semi urban area of Karachi, Pakistan to assess the effect of maternal morbidities on the weight of the newborn. We are following 400 pregnant women from the first trimester onwards until their delivery. The pregnant women are visited weekly to record any fever or respiratory symptoms during the past seven days, and are referred to the study site clinic for treatment of observed illnesses. Each symptom episode is defined as one or more days of a self-reported symptom (fever, cough, difficulty breathing, runny nose, sore throat, head ache, chills or myalgia) in a pregnant woman who was symptom free for three days before. So far, 288 pregnancies have concluded as live deliveries, 12 as still births and 31 as spontaneous abortions. We analyzed the data of 243 pregnant women whose newborns were weighed within 14 days of birth. The average age of pregnant women in our study was 24.1years and average weight of the pregnant woman was 56.1kg at the time of enrollment. Only 31% of the mothers had primary education or above whereas 38.3% had antenatal visits during their pregnancy. There were 51(21%) newborns with low birth weight (< 2.5 kg), whereas 192(79%) had normal birth weight (>= 2.5kg). In pregnant women who had a low birth weight baby, the average episodes of fever, cough, headache and myalgia were 1.7, 2.3, 4.3, and 4.3 per women respectively. In pregnant women who had a normal birth weight baby, the average episodes of fever, cough, headache and myalgia were 1.7, 2.1, 5.2 and 4.6 per women respectively. The results of this study will help identify the degree to which maternal respiratory illnesses during pregnancy are a risk factor for infant's low birth weight.

1369

MOLECULAR DETECTION OF HUMAN METAPNEUMOVIRUS ON NASOPHARYNGEAL SWABS COLLECTED FROM OUTPATIENTS WITH ACUTE RESPIRATORY TRACT INFECTIONS FROM MBAGATHI DISTRICT HOSPITAL, KENYA IN THE YEAR 2008

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Human metapneumovirus (hMPV) is a leading cause of acute respiratory tract infection in children the elderly and immune compromised persons. In Kenya the extend of hMPV infections in the population remains is unknown. A retrospective study was conducted in the year 2008 in outpatients from ≥2 months of age presenting at the outpatient department of Mbagathi District Hospital for acute respiratory infection. Nasopharyngeal swabs were systematically tested for human metapneumovirus and other respiratory viruses, using real time reverse transcriptase PCR. Epidemiological and clinical characteristics of hMPV-infected children were studied and compared to those of patients with respiratory syncytial virus (RSV) and other viral infections. A total of 498 patients were enrolled in this study. Viral investigations detected a total of 271 viruses. Out of these, 77(15.5%) were hMPV infections, 78(15.7%) Seasonal FluA, 60 (12%) Seasonal Flu B, 13 (2.6%) Panenterovirus, 36(7.2%) Para Influenza viruses and 6(1.2%) RSV infections. Human metapneumovirus infections were higher in males 43(55%) than females 34(45%), and predominantly in children ≤5yrs (97%), only 2(3%) aged between 6-9yrs. The hMPV infection had peak in January-February, and was uncommon after March. Most of the patients infected with hMPV were under 1 year of age and cough (100 %) and difficulty in breathing (75%) were the predominant diagnosis in these patients with clinical symptoms of a lower respiratory tract infection. The severity of the disease was similar to those of RSV patients. These results highlight that hMPV plays an important role in acute respiratory tract infections especially in children. Rapid detection to identify specific viral pathogens causing respiratory tract infections in the wider Kenyan population could aid in patient management.

1370

MOLECULAR CHARACTERIZATION OF HUMAN ENTEROVIRUS 68 ISOLATED IN KENYA DURING 2008 TO 2010

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Human enterovirus 68 (HEV-68) is a rarely detected viral pathogen associated with acute respiratory illness. It is unique among enteroviruses because it shares common biological properties with rhinoviruses. The

virus was first isolated in California, USA in 1962 and has ever since been identified almost exclusively in respiratory samples. HEV-68 infection is associated with several disease manifestations ranging from mild respiratory illnesses to severe acute lower respiratory tract infections including pneumonia, wheezing and bronchitis. During the period 2008 to 2010 an upsurge in the number of clusters of acute respiratory illness associated with HEV-68 was reported in many parts of the world including Asia, Europe and the United States. Human respiratory enteroviruses have not been well characterized in the East African region. We sought to molecularly characterize HEV-68 isolated in Kenya in 2008 to 2010 in order to understand their genetic diversity. A total of six (6) isolates were analyzed. Viral RNA was extracted followed by RT-PCR amplification of VP1 capsid protein coding gene. PCR amplicons were sequenced and the resulting sequences compared to those of Fermon prototype strain and previously characterized strains from other countries. Pair-wise comparison of VP1 sequences of Kenyan HEV-68 isolates revealed 87.2-99.5% nucleotide identity. The Kenyan HEV-68 strains shared 86.0-88.4% and 91-99% nucleotide identities respectively, when compared to Fermon and previously characterized strains reported in Gen Bank. Multiple sequence alignment of VP1 sequences of Kenyan isolates with Fermon revealed 12 amino acid substitutions and one deletion in five of the isolates and 11 amino acid substitutions. Phylogenetic analyses revealed five of the Kenyan isolates clustered closely to HEV-68 strains which circulated in New York, USA and Yamagata, Japan; while only one clustered with those that circulated in the Netherlands, Europe. All the Kenyan isolates clustered away from Fermon indicating divergence from the prototype strain. These findings suggest HEV-68 strains isolated in Kenya during the period 2008 to 2010 were generally similar to those detected in other parts of the world. Majority of the Kenyan isolates were however more closely related to those detected in the United States and Japan. Surveillance and constant monitoring of HEV-68 is important in understanding their evolutionary dynamics.

1371

MICRORNAS AS BIOMARKER FOR ACTIVE TUBERCULOSIS INFECTION IN IMMUNOCOMPETENT AND IMMUNODEFICIENT

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One of the most important priorities for tuberculosis control is the accurate diagnosis of individuals with active, infectious TB. This enables prompt treatment that both interrupts TB transmission and cures patients. Circulating nucleic acids (CNAs), including miRNAs present in serum, may serve as potential biomarkers for diagnosis and follow up in active and latent TB infection in immunocompetent and immunodeficient . The study enrolled consented participants, from 2 sites in Italy and 2 site in Africa (Tanzania and Uganda).Participants enrolled included healthy controls (HC), subjects with active TB (PTB), PTB with HIV and latent TB (LTBI). To minimize individual variation; sera from 10 participants from each category were pooled. miRNAs profile were measured using Taqman low density arrays qRT PCR. A Student's t test was used to compare mean concentrations of miRNAs with STATA 11. 672 miRNAs were analysed, 47 wmiRNAs were significantly up or down regulated and observed to be common in the active TB and HCs from both geographic region ($P<0.05$). 50 miRNAs were significantly expressed in active TB compared to LTBI; 37 and 11 miRNAs were up and down regulated respectively. Analysis performed following validation on single patients confirmed 4 common miRNAs from both the European and the African participants. Whereby: 7 miRNAs (3 European and 3 African specific and 4 common) were identified as discriminatory biomarkers for active TB disease. In conclusion, results from this study suggest that change in miRNAs expression levels plays a vital role in TB pathogenesis and could be biomarkers for TB diagnosis.

1372

PREVALENCE AND RISK FACTORS FOR LATENT TUBERCULOSIS INFECTION IN MILITARY PERSONNEL IN PERU

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Tuberculosis continues to be a global threat to public health. About of one-third of the world's population has latent tuberculosis infection (LTBI), with the highest rates generally in developing countries. Understanding the risk factors for LTBI is crucial for tuberculosis control. Populations in closed settings, such as military bases, are often at particularly high risk. Close contact with tuberculosis cases has been used to assess the risk of LTBI as well as active tuberculosis. Use of the tuberculin skin test (PPD) can identify persons with LTBI, but is often considered not useful to assess recent exposures in developing countries because it is assumed that the vast majority of persons are positive. We explored the prevalence and risk factors for LTBI in students and cadets in two military academies of the Peruvian Armed Forces. Participants were interviewed and received PPD placement. A total of 621 participants were enrolled, with a mean age of 19 years and 80% were male. Of 608 participants who returned for PPD evaluation, 118 (20%) were positive, with increasing prevalence with age; the multivariate logistic regression analysis showed that for every year of increased age the odds ratio for LTBI increased by 33%. Gender, area of birth and present residence, and close contact with tuberculosis cases or with relatives/friends with tuberculosis were not associated with LTBI. Despite the assumed high burden of tuberculosis in developing countries and closed settings, a minority of persons in our study were PPD positive and close contact with tuberculosis cases was not a risk factor for LTBI. However, increasing age was a good predictor for LTBI and should form the basis for targeted control efforts. We assume, but cannot be certain, that our study population is representative of the general population of Peru.

1373

INCIDENCE OF RESPIRATORY TRACT INFECTIONS AMONG PASTORALISTS BEFORE AND AFTER THE INTRODUCTION OF PCV-10 VACCINATION IN RURAL NORTHERN KENYA

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Worldwide, pneumonia is the top killer of children under five years old, taking the lives of 1.2 million children every year. Developing countries bear the highest burden of childhood mortality, and 30,000 of these yearly childhood pneumonia deaths occur in Kenya. To curb the effects of this killer disease, the Kenyan Ministry of Health introduced the 10-valent pneumococcal conjugate vaccine (PCV) to the routine immunization schedule in late 2010. Despite the introduction of PCV-10 to the schedule, however, inadequate access to vaccination in some parts of Kenya suggests immunization rates are too low to induce herd immunity in these communities. To explore the relationship between immunization for PCV-10 and respiratory infection rates, we conducted a retrospective study of

vaccination and outpatient records from two rural dispensaries who service pastoral populations in Laikipia county, Kenya. We found that PCV-10 coverage is very low, with only 33.3% of children receiving the first dose of PCV-10, 13.6% the second dose, and 6.15% the final dose. T-tests with unequal variance using Satterthwaite's degrees of freedom show no significant decrease in pneumonia incidence after vaccination began in Laikipia in March 2011 at either dispensary (Dispensary A: t (34.845) = 0.081, p = .468) and (Dispensary B: t (39.889) = 1.068, p = .146). However, the variance of pneumonia cases in months of high vaccination coverage is significantly lower than the variance of cases during low vaccination months (F (7,38) = 0.266, p = .037), suggesting that if great numbers of children are vaccinated with PCV-10, significant reductions in pneumococcal disease rates may occur.

1374

EVALUATION OF ACCEPTABILITY AND PERFORMANCE OF STOVE OPTIONS FOR REDUCING HOUSEHOLD AIR POLLUTION IN RURAL WEST KENYA

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Relationships between household air pollution (HAP) and risk of key diseases suggest low levels are needed to realise most of the health benefit; furthermore, achieving low levels requires that households are willing to use effective stoves for all or most needs. This study aims to identify whether one or more solid fuel stoves are capable of both meeting user needs and delivering low HAP, and hence suitable for intervention studies and scaling up. The study was conducted in west Kenya using mixed methods. Candidate stoves were required to demonstrate ≥ 40% reduction in PM2.5 emissions in USEPA tests. A cooking demonstration assessed user views on those most suitable for local needs: the 6 best (2 rocket, 1 chimney rocket, 3 fan-assisted) were then evaluated in a cross-over design in 43 homes. Following baseline measurement of kitchen concentrations (CO, PM2.5), personal women (cook) and youngest child (< 5 yr) CO, and stove use with stove use monitors (SUMS, each home used one stove type for 2 weeks, with repeat assessment in the final 48 hrs. This cycle repeated until all homes used at least 5 stoves. Qualitative interviews at baseline and following use of each stove assessed user views and reasons for multiple stove use. Focus groups (FG) explored user views in comparing all stove types. Initial (Round 1&2) results for kitchen and personal HAP show reductions for all stove types, but not to the low levels sought. SUMS data show multiple stove use occurred, and high kerosene lamp emissions may also help explain post-intervention HAP levels. Qualitative findings indicate preference for the new stoves, women reporting smoke reduction and finding them cleaner, more fuel efficient and easy to use. However, a number of stove improvements are suggested which could reduce multiple stove use. For most women, stove cost is reported as a barrier, but the FGs identified ways these could be made more affordable and marketed. Full results (to be presented) will help guide technology development and adoption to help deliver substantive health benefits at scale.

1375

ANTIBIOTIC USE IN AN INFLUENZA-LIKE ILLNESS COHORT IN PERU, 2009-2011

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Influenza-like illness (ILI) affects 20% of the global population annually. Although over 95% of ILI cases are thought to be viral, several studies have shown that patients and physicians often confuse ILI with respiratory infections caused by bacteria, prompting antibiotic prescription and inducing antibiotic resistance. To date, there has been no study on the use of antibiotics in persons with ILI in Peru. Therefore, we collected data on medication use, both prescribed and over-the-counter, from a multi-site ILI cohort study in four distinct ecological regions of Peru during 2009-2011. We compared antibiotic use associated with region, gender, age, presence of co-morbidities, and use of other medications in the preceding 30 days by chi-square analyses performed in EpiInfo. Data were collected on 6,790 cases of ILI, of whom 53% were female, with a median age of 14.7 years (range newborn-108). Overall, 92% of study participants took some medication for their ILI, of which 13% were prescribed by a physician. Co-morbidities and previous medication use were reported in 15% and 24%, respectively. Virtually all of those who took prescribed medications also took over-the-counter ones. Antibiotics comprised 27% of all medications, of which 51% were prescribed and 62% were penicillin drugs. Interestingly, despite data showing that approximately 20% of ILI cases in the cohort are influenza, no person took an anti-influenza drug, although these are not readily available on the market or in private clinics in Peru. The proportion of antibiotic use was higher than all other drugs taken for ILI. Prescription drugs, including antibiotics, are clearly frequently taken by persons with ILI in Peru. Although the etiologic agent is unknown in the majority of cases, the results almost certainly demonstrate an overuse of antibiotics for ILI, despite universal recommendations against the use of antibiotics for this syndrome. Increased availability of on-site diagnostics and dissemination of guidelines on the management of ILI at healthcare centers could improve this situation.

1376

XPERT MTB/RIF FOR THE DIAGNOSIS OF TUBERCULOSIS IN CHILDREN - A SYSTEMATIC REVIEW AND META-ANALYSIS

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In 2011, the WHO recommended Xpert MTB/RIF for the diagnosis of tuberculosis (TB) and MDR TB in all age groups despite a lack of pediatric data at that time. We conducted a systematic review to assess the diagnostic accuracy of Xpert MTB/RIF for pulmonary TB (PTB) in children. We performed database searches for relevant studies in all languages through April 2013. We included randomized-controlled, cross-sectional, and cohort studies involving children (< 15 years) with presumed TB. We extracted data separately for expectorated sputum (ES), induced sputum (IS), nasopharyngeal aspirates (NPA), and gastric aspirates (GA). We performed meta-analysis to determine pooled sensitivity and specificity. We included 10 studies in PTB. Five studies (41.7%) were conducted in

low or lower middle-income countries. Against a reference standard of culture, pooled sensitivities were 69% (95% Credible Interval 55-81) for ES and IS combined (7 studies) and 75% (59-90) for GA (5 studies). In HIV-infected children, sensitivity was 77% (60-89) in ES/IS versus 59% (44-72) in HIV-uninfected children. Sensitivity in ES/IS in children aged 0-4 was 57% (36-74) versus 83% (68-92) in children aged 5-15. Pooled specificity was >95% in all subgroups assessed using culture as a reference standard. In children with smear positive disease, pooled sensitivity was 96% (90-99) for ES and IS and 95% (83-99) for GLA. Pooled sensitivity in smear negative disease was 76% (58-90) for ES/IS and 78% (59-92) for GLA. As Xpert MTB/RIF is being rolled out in TB high burden settings it becomes available for children as an alternative to smear microscopy. Xpert MTB/RIF is highly specific for TB in children. However, sensitivity estimates are estimated with poor precision due to the sparse data available. There is a greater need for pediatric studies of Xpert to support guidelines for use in this population.

1377

COMPARISON OF NASOPHARYNGEAL SWABS COLLECTED FOR PNEUMOCOCCAL COLONIZATION AND NASAL SWABS IN THE IDENTIFICATION OF VIRAL RESPIRATORY INFECTIONS IN PERU

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We sought to determine agreement in detection of respiratory viruses using RT-PCR testing between two different types of samples collected on the same day: nasal swabs preserved in viral transport medium (NS) and nasopharyngeal swabs preserved in skim milk-tryptone-glucose-glycerol [STGG] media (NP). Samples were collected as part of a prospective household-based cohort study of Andean children aged less than 3 years. Nasal swabs were collected during episodes of acute respiratory illness for identification of respiratory viruses including influenza, human metapneumovirus (MPV), respiratory syncytial virus (RSV), human rhinovirus (HRV), parainfluenza virus 3 (PIV) and adenovirus (AdV). NS used a Dacron swab placed into each nostril sequentially, rotated beneath the turbinates, and placed into viral transport medium, which was then aliquoted into lysis buffer and stored at -80C. NP swabs were collected on a monthly basis to study colonization with *Streptococcus pneumoniae*. NP used a Rayon wire-handled swab placed through one nostril into the posterior nasopharynx, rotated for 5 seconds, placed into STGG and stored at -80C. A random sample of paired NP and NS samples collected from the same child on the same day was selected. Nucleic acid was extracted and tested for respiratory viruses by real-time monoplex RT-PCR. We evaluated the agreement between NP and NS samples in viral detection using the kappa coefficient and compared viral loads in NP and NS samples using RT-PCR cycle thresholds (CT). We studied 260 paired NP and NS samples. The kappa coefficient between NP and NS virus testing results was 0.70 (AdV); 0.87 (RSV); 0.88 (influenza); 0.92 (PIV3); 0.96 (HRV); and 0.97 (MPV). Median CT values were not statistically different between NP and NS samples across most respiratory viruses, except for influenza and RSV for which CTs were slightly lower in NP than in NS (all $p<0.05$). The agreement between NS and NP samples was very high, indicating NP samples could be used as a single, efficient collection strategy for field studies of both respiratory viruses and bacteria.

1378

LOPHOMONAS SP. IN RESPIRATORY TRACT SECRETIONS IN HOSPITALIZED CHILDREN WITH PNEUMONIA AND *BORDETELLA PERTUSSIS* CO-INFECTION

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Lophomonas sp. is a protozoan that is found in the digestive tract of cockroachs: *Periplaneta americana* and *Blatta germanica*. There are few reports of this emerging protozoan infection in humans, mainly affecting the lower respiratory tract in patients with severe lung disease. *Lophomonas sp.* has recently been reported in patients with asthma, as well as the discovery of protozoa in the respiratory tract of children with pneumonia. The aim of the study was to investigate *Lophomonas* in respiratory samples of children with pneumonia and in patients with a clinical diagnosis of pertussis, treated at the National Institute of Child Health, national reference center for pediatric diseases in Lima, Peru, in the period January to December 2012 and from January to March 2013. 558 samples were worked: 471 from tracheal aspirate, 40 from bronchoalveolar lavage and 47 from nasopharyngeal aspirate. This last group corresponding to children with a clinical diagnosis of pertussis. *Lophomonas* was found in 17/558 (3.04%) samples of children with pneumonia, six of them were diagnosed with pertussis. A sample with *Lophomonas sp.* and *Bordetella pertussis* coinfection was found out. In conclusion, it is necessary to search for *Lophomonas sp.*, emerging protozoan upper and lower respiratory infections, mainly in children with pneumonia and in patients diagnosed with pertussis.

1379

ESTABLISHMENT AND SUCCESSES OF THE UGANDA NATIONAL VIRAL HEMORRHAGIC FEVER SURVEILLANCE PROGRAM AND HIGH-CONTAINMENT LABORATORY, 2010-2013

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Uganda is endemic for viral hemorrhagic fevers (VHF) and other zoonotic diseases. In July 2010 the Viral Special Pathogens Branch, CDC, the Uganda Virus Research Institute (UVRI), and the Ministry of Health established a first of its kind National VHF surveillance program. In addition, a permanent high-containment laboratory was established at UVRI. This lab serves as the national VHF reference laboratory, and an East Africa regional resource. The laboratory can perform real-time PCR, IgM, IgG and antigen capture ELISA for Ebola, Marburg, Rift Valley fever, and Crimean-Congo hemorrhagic fever viruses. To date, suspect VHF samples from over 20 districts in Uganda, and 5 East and Central African countries, have been sent for rule-out testing. The program has tested over 2000 samples from human surveillance, serosurveys, primates, livestock, and VHF outbreaks. 1364 samples have been tested for surveillance and outbreak activities, resulting in confirmation of 5 independent filovirus outbreaks. Four Ebola outbreaks have occurred: two in Luwero District (2011, CFR=100%; 2012, CFR=57%; both Sudan virus), one in Kibaale District (2012, CFR=71%; Sudan virus), and one in Isiro, DRC (2012, CFR=54%; Bundibugyo virus). One Marburg outbreak occurred in Kabale, Kamwenge, and Ibanda districts in 2012 (CFR=58%.). Testing was completed for a national Uganda serosurvey of 587 human blood

samples looking for evidence of past infection with Ebola, Marburg, RVF, and CCHF. The program has also tested 244 primate and livestock samples for VHFs, including 204 samples from the Karamoja region where 35% were positive by IgG for CCHF, showing evidence of actively circulating CCHF virus in Uganda. The successes of this program show how having a functional, comprehensive, and timely VHF surveillance system in Uganda, and East Africa, greatly contributes to limiting the extent of outbreaks through early detection and response. This program also advances the knowledge of other high-hazard pathogens of international concern in Uganda, and the region, and should serve as a model for further expansion throughout Africa.

1380

RE-EMERGENCE OF BUNDIBUGYO VIRUS AFTER A FIVE YEAR HIATUS -- ISIRO, THE DEMOCRATIC REPUBLIC OF THE CONGO, 2012

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On August 16, 2012, two patients in Isiro Health Zone, The Democratic Republic of Congo (DRC) tested positive for Bundibugyo virus (BDBV), identifying the second Ebola Hemorrhagic Fever (EHF) outbreak attributed to BDBV – the first occurring in Uganda in 2007. An international response was immediately launched to control the outbreak. Patient epidemiologic and clinical data were collected via a standardized case report form. A field laboratory tested blood samples by RT-PCR, and serology diagnostics were performed in Uganda. Cases were classified as suspect (clinical criteria), probable (suspect plus epidemiologic criteria), or laboratory confirmed. Bivariate data analysis was used to evaluate cases and non-cases for predictors of BDBV infection, and cases for predictors of death. A total of 36 confirmed, 16 probable, and 7 suspect EHF cases were identified, with 133 initially suspected cases ruled out. Among the 52 confirmed and probable cases, the case fatality rate (CFR) was 53.8%, age range was 0 – 70 years (median=40 years), 76.9% were female, and 25.0% were healthcare workers. Among all patients evaluated for EHF, factors significantly associated ($p<0.05$) with being a case were female gender, fever, vomiting, diarrhea, fatigue, conjunctivitis, difficulty swallowing, difficulty breathing, hiccups, and anorexia. Patients with a cough were significantly less likely to be a case. Among EHF cases, symptoms significantly associated with death were hemorrhagic signs, cough, difficulty swallowing, difficulty breathing, conjunctivitis, and hiccups. Five unlinked chains of virus transmission were identified, indicating that EHF cases remained unidentified. June 1, 2012 was the earliest discovered case onset. Like the 2007 outbreak, this BDVB outbreak had a lower CFR than is seen with *Zaire ebolavirus* and *Sudan ebolavirus* species. The last confirmed case was isolated 57 days after outbreak detection. Prompt local and international efforts in field laboratory establishment, case finding, and case isolation were crucial to the successful containment of the outbreak.

1381

ROTAVIRUS INFECTION IN CHILDREN IN A RURAL COMMUNITY IN PISCO, PERU

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Rotavirus is the leading cause of severe diarrhea in children under 5 years of age worldwide, causing up to 50% of childhood hospitalizations for diarrhea in industrialized countries, and an even higher proportion in developing countries. However, data on rotavirus transmission rates in the community are much more sparse, especially in developing countries. Community-based data will be vital in assessing the effectiveness of rotavirus vaccination as the vaccine becomes more widely employed. We used data and samples available from a study on water quality to explore the prevalence of rotavirus infection in rural communities outside the town of Pisco, Peru. The study was conducted in 2010, one year after rotavirus vaccination was introduced in Peru's national immunization program, with reported rotavirus vaccine coverage in Ica region at the time of 64%. A convenience sample of 192 houses was selected and stool samples taken from one child age ≤ 5 years old from each household. Stools were tested for rotavirus by real time RT-PCR according to CDC guidelines. Of the 192 children enrolled, 54 (28%) were rotavirus infected. The proportion of rotavirus-infected children did not differ significantly between children who did and did not report an episode of diarrhea in the preceding two weeks: 11/32 (33%) and 43/160 (27%), respectively. The median age of the rotavirus-positive children was 24.5 months (range 5-48 months) and 50% were male. Unfortunately, because the original aim of the study was not oriented toward rotavirus, no specific rotavirus vaccination history on each child was taken. The finding of frequent rotavirus infection in children who did not recently suffer diarrhea suggests that additional factors, such as infectious dose, underlying co-infections or morbidities, or genetic predisposition are involved in producing clinical disease due to rotavirus infection. The results also provide baseline data useful for future assessment of rotavirus vaccine effectiveness in Peru.

1382

NIAKHA VIRUS: A NOVEL MEMBER OF THE FAMILY *RHABDOVIRIDAE* ISOLATED FROM PHLEBOTOMINE SANDFLIES IN SENEGAL

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Members of the family *Rhabdoviridae* have been assigned into eight genera but many remain unassigned. Rhabdoviruses have a diverse host range that includes terrestrial and marine animals, invertebrates and plants. Transmission requires arthropod vectors such as mosquitoes, midges, sandflies, ticks, aphids and leafhoppers, in which they replicate. Here we characterize Niakha virus (NIAV), a previously uncharacterized rhabdovirus isolated from phebotomine sandflies in Senegal. Analysis of the 11,124 nt genome sequence indicates that it encodes the five common rhabdovirus proteins with alternative ORFs in the M, G and L genes. Phylogenetic analysis of the L protein indicate that NIAV's closest relative is Oak Vale rhabdovirus, although still so phylogenetically distinct that it may be not classified as a member of the eight recognized *Rhabdoviridae* genera. This observation highlights the vast, and yet not fully recognized diversity, of this family, some members of which could potentially jump species boundaries in the future.

1383

EASTERN EQUINE ENCEPHALITIS VIRUS: REEMERGENCE AND EXPANSION IN THE NORTHEASTERN UNITED STATES

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Eastern equine encephalitis (EEE) virus is the most deadly mosquito-borne pathogen in North America with an estimated human case fatality rate of 35 to 75%. EEE virus activity is most common in and around freshwater hardwood swamps in the Atlantic and Gulf Coast states and in the Great Lakes region, where the primary mosquito vector *Culiseta melanura* resides. Since the discovery of EEE virus in the 1930s, outbreaks in temperate regions have been sporadic, both temporally and spatially, highly focal, and largely unpredictable. However, over the last decade, we have witnessed a sustained resurgence and change in dynamics of EEE virus activity within long-standing foci in the northeastern U.S. and unprecedented northward expansion into new regions where the virus had been historically rare or previously unknown, including northern New England and eastern Canada. This has resulted in severe disease in humans (46 cases with 16 fatalities) and domestic animals (173 cases). The factors responsible for reemergence of EEE virus are largely unknown but are likely complex reflecting ongoing changes in the ecology and epidemiology of this virus. Long-term changes in land-use, including wetlands restoration and suburban development, and increases in human population density near critical habitats may be important components. Weather conditions associated with climate change are also likely to be contributing factors. These include mild winters, hot summers and extremes in both precipitation and drought that increase vector abundance and distribution, elongate the virus transmission season, and increase the intensity of virus transmission by increasing the frequency of blood feeding and rate of virus replication in mosquitoes. These and other underlying factors associated with the introduction, amplification, persistence, and range expansion of EEE virus in the region including: 1) vector mosquito abundance and distribution that drive viral amplification and spillover into human and equine populations, 2) species-specific mosquito-avian interactions that favor amplification, 3) virus titers in primary and secondary mosquito vectors, and 4) genetic variation in regional EEE virus strains that provide evidence for local overwintering, evolution and extinction of EEE virus strains, with periodic reintroduction from southern sources, will be examined.

1384

EMERGING PATHOGENS IN MULTIPLE BAT SPECIES IN MADRE DE DIOS, PERU: *LEPTOSPIRA* AND PARAMYXOVIRUSES

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In recent years, bats have attracted considerable attention as hosts of emerging and other pathogens relevant to public health. We trapped bats and harvested their tissues for analysis near seven communities in the Madre de Dios Region in the southern Amazon basin of Peru as part of a study to explore the impact of anthropogenic habitat perturbation in the region (the building of the Peruvian interoceanic highway) on the distribution of reservoirs and pathogens. Bat kidneys were tested for *Leptospira* by PCR using primers that amplify 16S rRNA. Spleens were tested for paramyxovirus by nested PCR targeting the conserved motifs of the polymerase pol gene. A total of 432 bats from 24 different genera were captured, of which 32 (7%) were positive for *Leptospira*. All positive bats belonged to one of nine genera of the family *Phyllostomidae*, including the genera *Trachops* and *Lophostoma*. Twenty-six (81%) of the *Leptospira* positive bats were adults, while age could not be determined in the remaining 6 (19%). Infected animals were identified in 6 of the 7 sites sampled. Sequencing of PCR products is underway to identify the specific species of *Leptospira* implicated. Paramyxovirus testing was performed on 263 bats, of which 3 (1%) were positive. All 3 positive bats were adults of the *Sturnira lilium* species collected in one location in Iberia District. Sequence analysis placed the paramyxoviruses in the Avulavirus or Rubulavirus genera. Avulaviruses are known to date only to infect birds, while rubulaviruses such as Tioman, Mapuera, and Menangle have been described in fruit- and insect-eating bats, making Rubulavirus the more likely genus implicated here. Of note, rubulaviruses have been associated with encephalitis and influenza-like illness in humans. This is the first report of *Leptospira* infection in *Trachops* and *Lophostoma* in Peru, as well as of paramyxovirus infection in any bat in Peru, expanding our understating of the host and geographic range of these potentially emerging pathogens. Testing for other pathogens, including coronaviruses, is underway.

1385

AGE-STRATIFIED SEROLOGICAL SURVEY OF SYLVATIC CHIKUNGUNYA VIRUS IN NONHUMAN PRIMATES IN SENEGAL

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Sylvatic chikungunya virus (CHIKV) has been isolated in Senegal over the past 50 years. Until recently, virus isolation was predominantly from mosquito collections, and the virus was only isolated from nonhuman primates (NHP) after opportunistic capture. Here we present an age-stratified serological survey of CHIKV in three NHP species in Senegal and calculate forces of CHIKV infection for each species. African green monkeys (*Chlorocebus sabaeus*), patas monkeys (*Erythrocebus patas*), and Guinea baboons (*Papio papio*) were collected in the dry season in each of three years (2010-2012) from in Kedougou, Senegal. Primates were trapped, sedated, and bled and sera were tested for IgM by ELISA and IgG by PRNT. Ages of primates were quantified using pattern of tooth eruption and wear determined from photographs and dental casts; weight and other anthropometric measurements were also taken. Force of CHIKV infection was calculated using catalytic models with bootstrap confidence intervals. Random effect logistic models were fit to find associations between age, month of collection, and species with seropositivity. A total of 219 African green monkeys, 78 patas, and 440 baboons were collected between 2010 and 2012. Across all years, 66%, 36%, and 73% were seropositive for CHIKV antibody by PRNT50, respectively. Forces of infection were high, ranging from 0.13 per year (95% Confidence Interval [CI]: 0.07, 0.21) for patas in 2012 to 1.15 per year (95% CI: 0.81, 3.83) for African green monkeys in 2010. Logistic models with random effects for troop indicated age as significantly positively associated with PRNT50 positivity (Odds ratio [OR]: 1.030 (95% CI: 1.023, 1.036)), and significantly less positivity in patas compared to African green monkeys (OR: 0.73, (95% CI: 0.60, 0.89)). To our knowledge this is the first study of CHIKV transmission dynamics in its sylvatic reservoir host. It reveals very high forces of infection of CHIKV for all NHP species tested, with rates of seropositivity approaching 100% as primate age increases. Our demonstration of a CHIKV reservoir carries important consequences for individuals living or working in proximity to primate populations in Senegal, where CHIKV has the potential to cause major morbidity.

1386

HERPES SIMPLEX AS THE MOST COMMON CAUSE OF ENCEPHALITIS IN PERU

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Herpes simplex encephalitis (HSE) causes significant morbidity and mortality within developing countries, where the ability to diagnosis and treat HSE is limited. Our aim was to describe the clinical and cerebrospinal fluid (CSF) characteristics of HSE in patients presenting with symptoms of encephalitis to a network of hospitals in Peru. This was a prospective study of patients aged 28 days or older presenting with symptoms of encephalitis at nine Peruvian hospitals in three different geographical regions (coast, mountains and jungle) between February 2009 and March 2012. We enrolled patients presenting with clinical symptoms of encephalitis and diagnosis of HSE was confirmed by detection of HSV DNA in CSF using PCR. In this study 223 patients met clinical criteria for encephalitis and were included in the final analysis. Mean age of the patients was 5.41 years for children and 41.8 years for adults. 66.7% of children and 47.6% of adults were male. The mean time from onset of symptoms to hospital presentation was 9 days (range: 1 - 31). Headache, fever, seizures, neck stiffness, dystaxia, and nausea were the most common clinical symptoms. Seizures were more frequent in children (p=0.005), while headaches and neck stiffness were more frequent in adults (p=0.013 and 0.048, respectively). CSF was normal in 5.5% patients with abnormal glucose seen in 55.6%. Leukocyte counts, predominantly lymphocytes, were higher in adults than in children (p=0.031). HSV as determined by PCR was the etiology in 36 (16.1%) patients (21 adults ,15 children). The majority of HSE (88.9%) was due to HSV-1. HSV-2 was found in 2 patients from each age group. Co-infections with HIV were found in 5 (13.8%) adults and 3 patients also had *Cryptococcus neoformans* meningitis. HSV-1 was found to be the most common cause of encephalitis in Peru and emphasizes the need for improvements in diagnostic capabilities and acyclovir availability in developing countries.

1387

GENETIC CHARACTERIZATION OF NOROVIRUSES AMONG PERUVIAN ARMY RECRUITS IN THE AMAZON BASIN

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Norovirus is the number one cause of acute gastroenteritis worldwide, afflicting 21 million Americans and killing 200,000 children under five in the developing world each year. Understanding norovirus genetic variation may be important in the development of an effective vaccine. We assessed the various genotypes of norovirus circulating in a cohort of Peruvian military recruits under active surveillance for acute gastroenteritis between 2005-2011 in Iquitos, Peru. Stool specimens were collected from randomly selected participants, 200 with acute gastroenteritis and 200 healthy controls, and tested for norovirus genogroups GI and GII by real-time RT-PCR. Positive samples were genotyped by sequencing the C region of the capsid gene. Sequence fragments were aligned and compared to norovirus sequences available in the GenBank database. Norovirus was detected in 40/360 (11.1%) samples, 26/184 (14.1%) cases and 14/176 (8.0%) controls (the epidemiologic and clinical significance of these findings are discussed in a companion abstract). Of the 25 noroviruses that could be genotyped (18 from cases and 7 from controls), 11 were GI and 14 were GII. The predominant GI genotype was GI.4 (7 persons), followed by one each of GI.1, GI.3, GI.5, and GI.7. The predominant GII genotype was GII.4 (6 persons), followed by GII.17 (2 persons) and one each of GII.5, GII.6, GII.14, GII.15, and GII.16. Of the four GII.4 positive cases that could be further differentiated by variant, all were GII.4 Den Haag (2006b). The sample size was too small for meaningful statistical analysis, but the GII.4 genotype was the most prevalent genotype identified in cases, and it was not identified in controls. Multiple norovirus variants circulated in both cases and controls in this study, without other obvious associations with pathogenicity. Further research is needed to explore the possible clinical significance of the numerous variants of norovirus and to guide vaccine development.

1388

RODENT SPECIES AND THEIR CORRELATION WITH HUMAN SEROPOSITIVITY FOR ZOONOTIC INFECTIONS IN GHANA

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Rodents serve as reservoirs and/or vectors for several human infections which account for high morbidity and mortality in Africa. The remarkable expansion of human population has brought them into increasing contact with these mammals, thereby disrupting their habitats and increasing opportunities for disease transmission. To investigate possible risk factors for exposure to some of these pathogens, 764 small mammals were collected from ten communities in Ghana together with 657 human sera from healthy adults living in the same communities. Rodents were captured by setting Sherman collapsible traps along marked lines in fields (outdoors) and houses (indoors) totaling 9,269 night traps for three consecutive nights. The small mammals caught constituted ten genera of which whole blood of two rodents (0.3%) *Mus (Nannomys) sp.* tested positive for arenaviruses and one kidney tissue from Croccidura sp tested positive for *Leptospira* by conventional polymerase chain reaction (PCR). All rodent lung tissues were negative for Hantaviruses (Dobrava and Puumala serotypes). Using an in-house enzyme-immunoassay (ELISA), human serum showed evidence of arenavirus antibodies in 34 samples (5%). Antibodies to Puumala and Dobrava serotypes and Leptospirosis were also detected in 11%, 12% and 21% respectively with commercial kits. The occurrence of immunoglobulin G (IgG) antibodies to Dobrava and Puumala serotypes was more common in females (54%) than in males whereas the opposite was observed for Lassa virus (LASV) and Leptospirosis (52%). Human exposure to zoonotic infections was observed to cut across all age groups. Seropositivity was highest for anti-LASV at site 7 (29%), anti-hantavirus (Dobrava serotype) at site 10 (26%), and anti-*Leptospira* at site 8 (19%) located in the Eastern, Brong Ahafo, and Northern Regions respectively. Fifty six individuals had been exposed to more than one of the rodent-borne infections tested whereas 208 had been exposed to only one type of infection. The known reservoirs of the different pathogens that were tested in the human sera were captured in most of the study sites but human exposure could not be linked to their presence. This study suggests that 40% of residents in rural farming communities in Ghana have measurable antibodies to at least one rodent-borne disease (LASV, hantavirus, or Leptospirosis), which is not surprising given the ubiquitous presence of rodents in subsistence farming communities.

1389

PHYLODYNAMIC AND PHYLOGEOGRAPHIC PATTERNS OF EASTERN EQUINE ENCEPHALITIS VIRUS IN THE NEW WORLD

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Eastern equine encephalitis virus (EEEV) is a mosquito-borne alphavirus (Family; Togaviridae) of significant public and veterinary health importance throughout the Americas. EEEV exists as one antigenic complex but can be further classified into four lineages or subtypes based on serologic and phylogenetic analyses. Lineage I primarily consists of North American and Caribbean isolates, and lineages II-IV, which consist of Central and South America isolates, has been proposed to comprise a distinct species. Although EEEV is largely maintained locally (*in situ*), there is evidence of gene flow among and within countries. Lineage I strains utilize different hosts and vectors, and possess distinct neurovirulence characteristics, which are typically not observed among lineage II-IV isolates. This study aims to characterize EEEV genetic diversity, describe its molecular epidemiology, identify genetic determinants of EEEV emergence and virulence, and infer the phylodynamic and phylogeographic histories of EEEV. To this end, we performed a Bayesian analysis of EEEV complete genomes derived using next-generation sequencing. Results suggest differences in selective constraints and substitution rates among EEEV lineages. The data also suggests a Peruvian origin for EEEV, and that the virus spread to north eastern US prior to its expansion into other regions of the US. There is also evidence of significant gene flow within North America, suggesting that state level control measures would be inadequate for local elimination of the virus.

1390

EVASION OF HOST IMMUNE RESPONSE BY SEVERE FEVER WITH *THROMBOCYTOPENIA* SYNDROME VIRUS (SFTSV)

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Severe Fever with *Thrombocytopenia* Syndrome virus (SFTSV) is a novel member of the family Bunyaviridae, genus Phlebovirus. This virus was recently isolated from patients suffering from fever, thrombocytopenia, and hemorrhagic manifestations. SFTSV displays a mortality rate of 12% to 30% and direct human-to-human transmission has been reported. Due to the recent emergence of this pathogen, limited knowledge is available about the mechanism(s) involved in disease pathogenesis and the molecular mechanism(s) by which SFTSV suppresses innate immune responses. The type I interferon (IFN) responses are crucial for the development of antiviral immunity and therefore many pathogens have developed strategies that subvert these responses by blocking production of IFN or blocking IFN signaling. Indeed, type I IFN suppression has been described in other members of the genus. Likewise, we have observed that SFTSV infection inhibits type I IFN responses. SFTSV infection triggers the formation of cytoplasmic vesicles in which key components of the Type I IFN response, such as the cytosolic viral RNA receptor retinoic acid-inducible gene 1 (RIG-I) and its regulator the E3 ubiquitin ligase TRIM25, co-localize with viral proteins. Interestingly, the expression of the SFTSV nonstructural protein (NSs) is sufficient for the formation of vesicles and the co-localization of RIG-I and TRIM25 within them. SFTSV NSs not only co-localizes but also interacts with RIG-I and TRIM25. Furthermore, NSs inhibits the activation of the IFN-β promoter induced by virus infection or double-stranded RNA (dsRNA). Taken together, these data suggest that NSs inhibits type I IFN-mediated host protective innate immunity against viral infection by "sequestering" RIG-I and TRIM25 into the NSs-induced vesicles. Our studies provide mechanistic insights into viral pathogenesis and define a novel immune evasion strategy for subversion of host innate immune responses. This information will provide new targets for preventive and therapeutic interventions against SFTSV and other related pathogenic RNA viruses.

1391

NOROVIRUS GASTROENTERITIS AT A SKILLED NURSING FACILITY

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In January 2011, symptoms of nausea, abdominal cramps, and diarrhea were reported in a 250-bed skilled nursing facility. Investigations revealed that these symptoms were present in some residents and employees as far back as two days prior, but they were thought to be isolated cases. Upon realization that the cases reported could be an outbreak, immediate actions were put in place to contain the outbreak and to prevent its spread throughout the facility. Immediate actions that were taken included: restrictions of all residents with symptoms in their rooms; staff education that include nursing, environmental, therapists and other caregivers; increasing hand hygiene; restricting visitors to the facility. Sick visitors were asked to stay away; signs were posted on all floors; increasing frequency of cleaning of "high touch" surfaces; using bleach to clean high touch surfaces; notification of the County Department of Health; continued surveillance for immediate identification of new cases and monitoring of old ones; hydration protocol for affected residents to prevent debilitating effects of the virus; sending of samples to the County Department of Health for definitive identification; restrictions of affected employees from care areas; education provided by County Public Health Nurse on the first week of this outbreak. Overall, the outbreak involved over 70 facility residents and the following departmental staff: Nursing, Nutritional Services, Office and Maintenance. On the average, the symptoms lasted about 48 hours for each individual. The norovirus gastroenteritis transmission subsided once the control recommendations (above) were implemented within three weeks after the index case occurred.

1392

INFLUENZA A VIRUS IN SWINE FARMS FROM GUATEMALA: EVIDENCE OF ZOONOTIC TRANSMISSION FROM HUMANS

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In 2009 the emergence of the pandemic H1N1 (pH1N1) strain of influenza A virus (IAV) of potential swine origin, highlighted the need of surveillance of influenza virus in pigs. In Central America, Guatemala is the country with the largest pork production; however the circulation of IAV in the swine population has not been investigated in detail. The main objectives of this study were to determine the presence of IAV in the swine population in Guatemala and identify the circulating subtypes including pH1N1. Two nation-wide multistage random surveys for IAV were conducted. Nasal swabs and blood samples were collected from swine farms and backyard operations during October 2010 and from June to August 2011. Samples were collected from 171 herds in 2010 (n=500) and 136 herds in 2011 (n=499). Herd prevalence for IAV detected by rRT-PCR was 33% for both years. From rRT-PCR positives 4 viruses were isolated, based on their full genome sequences, 3 were fully pH1N1 and one a fully H3N2 seasonal human-like strain. Additionally, antibodies

against IAV were detected by ELISA with herd prevalences of 17.5 and 5.1% for 2010 and 2011 respectively. The H1N1 and H3N2 subtypes from different genetic clusters (swine and human-like) were detected by hemmaglutination inhibition assay. These results suggest that different IAV circulate in the swine population of Guatemala and that human-animal contact may play a role for the introduction of novel strains into the swine population. Global and local methods were used to establish if spatial correlation exists in the IAV positive swine farms from each year. This study is the first in Guatemala analyzing AIV prevalence and its distribution in swine farms from the country.

1393

DEVELOPMENT OF SYSTEM FOR THE APPLICATION OF ANTIRABIC VACCINES IN UKRAINE

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In order to develop and implement an effective program for rabies eradication in Ukraine, a collection of samples of pathological rabies-positive materials selected from 17 animal species and humans was founded in 2008 on the basis of regional veterinary laboratories of Ukraine. The collection is regularly updated and now includes 1,340 samples from all regions of Ukraine. We performed a Molecular-genetic study of the collection material using 156 pathological samples/probes from animals having rabies. We performed PCR tests and virus isolation with further sequencing. The study resulted in the determination of two genetic clusters and their clear geographical division in relation to river Dnieper. The genetic clusters' prevalence mapping for the last 5 years showed that cluster II isolates circulate in the regions with maximum rabies spread. Antirabic vaccine efficiency against the two cluster strains circulating in environment was evaluated on the stage of the work. Commercial vaccines obtained with the employment of the rabies virus vaccine strains SAD (Street-Alabama-Dufferin) and Wistar PM/WI were used for the evaluation. The study showed that all the vaccines were 30 % less effective for the cluster II rabies viruses than for the cluster I viruses. The performed research demonstrated genetic and antigen difference between environmental rabies viruses and the strains used for the vaccines production. The obtained results enable one to assume the low efficiency of antirabic vaccines against genetic cluster II rabies viruses as a potential reason for the high rabies prevalence in certain territories. The study results will be employed for the efficiency elevation of antirabic vaccine use in Ukraine on the basis of differential approach depending on vaccine immunogenic activity as well as geographical distribution of rabies virus strains. The performed work also points at the necessity of the development of new regional rabies virus vaccine strains in future.

1394

SEARCH OF ANTHROPURGIC REASONS FOR RABIES IN UKRAINE

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Rabies is an acute viral encephalomyelitis that affects wild and domestic mammals. Worldwide, human death due to rabies makes approximately 55,000 cases annually. Red foxes (Vulpes vulpes) are natural reservoirs of rabies virus in Ukraine. We used the monitoring and mathematical methods for this research. Despite considerable financial expenses on oral immunization of foxes and parenteral immunization of dogs and cats, considerable results have not been achieved in the fight against rabies in Ukraine. It is observed a tendency to increasing of rabies cases in dogs and cats which are the main source of rabies in people. In epidemic section in Ukraine, cats pose the highest hazard, as they are the basic source of infection for humans - 41.3 %, while the rates for dogs and foxes are 24.1 % and 20.7 %, respectively. The rest cases appear from other undefined contacts. When analyzing data, the most important epidemic route for rabies in Ukraine was defined. This could be depicted as follows - fox→cat→human. Over the last decade, nearly 10.5 thousand cases of rabies in cats were detected (average cats morbidity index in Europe is 13.2 %); herewith, 42 % of these cases were registered in Ukraine. The situation analysis based on the reports from veterinarians and veterinary service representatives in urban areas on quantity of fox bites in animals, showed that one of the reasons was the close location of fox inhabitation to urban areas, and their contacts with cats due to the common nutritive base - murine rodents. One more reason was a weak control over the execution of domestic animals' keeping rules, irresponsibility of their owners especially at suburban areas, and small percentage of cats vaccinated against rabies. For instance, 85 % of cats infected with rabies were kept by owners without timely provided vaccination against rabies. With intent to improve the situation with rabies in Ukraine, it is necessary to strengthen control over the rules for keeping domestic animals, increase responsibility of animals' owners for breaking the rules and ensure complete preventive vaccination of cats in endemic zones.

1395

APPLICATION OF GUANCID TO ENSURE BIOLOGICAL SAFETY DURING WORK WITH AUJESZKY'S DISEASE VIRUS

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The purpose of a research was to detect the optimal exposure time for the elimination of Aujeszky's disease agent and define Guancid disinfectant safe concentration for animals and humans. The Guancid active component is polyhexamethyleneguanidine hydrochloride. Pig embryo kidney (PEK) and young pig testicle (YPT) cell culture were cultured on well bottoms of 96-well microplate in the form of a monolayer. Different media (RPMI 1640, DMEM, GLA), series 07 bovine blood serum, and phosphate-buffered saline (PBS) were employed for the cell culturing. The "Clone-B" vaccine strain of Aujeszky's disease agent (ADA) was used as the control virus. In order to define the disinfectant antiviral effect, different concentration Guancid solutions in PBS were employed: 0.01; 0.03; 0.05; 0.1; 0.5; 1.0; 2.0; and 3.0 %. After the solution application to the wells, ADA viral suspension with the activity of 107TCD50/cm3 (a dose of virus that causes cytopathic effect in PEK and YPT cells in 24-28 hours without treatment with disinfectant). The virus-disinfectant interaction was performed within 1-60 minutes. Subsequently, the well content was applied onto the surface of cell monolayer to absorb the virus, which was not inactivated by the disinfectant. After 20-minute contact, the microplate was triply washed with PBS and filled with a supporting medium with 2 % of bovine blood serum and incubated. 16 wells with cell cultures were left without disinfectant as the control. Microscopy of wells was conducted twice a day. Results were assessed prior the moment of degenerative changes in the control wells of the microplate. Virus cytopathic effect on the cell cultures was determined at Guancid solution concentrations of up to 0.05 % and at 60 minute exposure. The disinfectant displayed this effect at 2 and 3 % concentrations and at 20 minute exposure. No virus or disinfectant cytopathic effect was detected after 30-minute exposure using Guancid solution concentrations from 0.1 to 1.0 %. It could be concluded that Guancid concentrations from 0.1 to 1.0 % and 30-minutes exposure should be used to ensure biological safety during work with ADA. The disinfectant could be employed for preventive disinfection in veterinary in the presence of animals and humans.

1396

GEOGRAPHIC DISTRIBUTION OF ZOONOTIC AND VECTOR-BORNE SELECT AGENTS IN KENYA: A SEROLOGIC SURVEY USING A SUBSET OF SERA SAMPLES COLLECTED THROUGH THE KENYA AIDS INDICATOR SURVEY (KAIS)

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Zoonoses are diseases and infections which are transmitted naturally between vertebrate animals and man. Vector-borne diseases are infections that are transmitted by the bite of an infected arthropod. Of all human infectious diseases, 61% are zoonotic while 75% of human Emerging Infectious Diseases (EID) are zoonotic. Out of these zoonoses, 33% have a human to human transmission. The dynamics of population growth and lifestyle increases the human-animal-vector encounters and as such exposing humans to zoonotic infections. The incidence and prevalence information of select zoonotic and vector-borne diseases in Kenya is either limited or unknown even though they have been documented to cause outbreaks. An assessment of exposure levels to select zoonotic and vector-borne agents, will determine the most at risk populations to infections as well as outbreaks and therefore target public health interventions. An assessment of co-infection between the select agents will determine what role do co-infections play in prognosis. A total of 15,853 blood samples were collected and analyzed during the 2007 Kenya AIDS Indicator Survey (KAIS). During data collection, serum from participants that consented to storage of their specimens for future testing were separated into several cryovials for processing, including a "storage vial" which was stored in -70°C for future testing. A nationally representative subset of the samples (1091 specimens) was selected and tested for Anthrax, Brucella, Chikungunya, Dengue, Rift Valley Fever, *Rickettsia* and *Leishmania* by IgG Enzyme Linked Immunosorbent Assay (ELISA). Preliminary findings give an indication of geographical hotspots in some regions in Kenya. Of the 1091 serum samples tested, analysis was done by province, residence (rural/urban) and by wealth quintiles. A Dengue, RVF and Richettsial analysis by province shows coast province to have the highest prevalence of antibodies against the 3 select agents followed by North Eastern. The least affected province is Rift Valley. In relation to residence, the rural dwellers were more affected than their urban counterparts. When analyzed by wealth quintiles, the lowest quintile was seen to have the highest prevalence, while the middle quintile had the lowest prevalence of antibodies against the select agents.

1397

CHARACTERIZATION OF NEUTRALIZING ANTIBODY RESPONSES FOLLOWING NATURAL PRIMARY INAPPARENT AND APPARENT DENGUE VIRUS INFECTIONS

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Dengue virus is the most significant arthropod-borne virus of humans. Primary dengue infection induces both neutralizing antibodies towards the infecting dengue serotype and cross-reactive non-neutralizing antibodies to other dengue serotypes. The theory of antibody dependent enhancement predicts that cross-reactive antibodies enhance secondary dengue infections, thus resulting in severe disease. During a pediatric fever surveillance cohort study in Colombo, Sri Lanka, sera samples were collected at regular yearly intervals and during and soon after dengue fever episodes. Here, we report on studies that were conducted, using prospectively-collected samples from that cohort, to compare the quality and quantity of dengue-specific antibodies in children with inapparent and apparent infection. Both dengue-specific IgG levels and neutralizing antibody responses induced by primary inapparent and apparent infections were similar. We followed primary dengue cases for up to two years to hone in on the specifics of neutralizing antibody decay over time. Primary infections induced broad-neutralizing antibodies that gradually became monospecific to the infecting serotype over time. The presence of dengue-specific IgM was correlated with broad neutralization. In children exposed to secondary infections, we observed that children with pre-existing monospecific neutralizing antibody responses were more likely to develop fever upon a secondary dengue infection than children with broadly neutralizing antibody pre-existing responses. In all, our findings provide unique insight about development and timing of the neutralizing antibody response following natural primary dengue infection and how such a neutralizing antibody response may influence fever outcome upon secondary infection.

1398

MUTATIONS THAT MODULATE DENGUE VIRUS "BREATHING" HAVE A SIGNIFICANT IMPACT ON SENSITIVITY TO ANTIBODY-MEDIATED NEUTRALIZATION

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Flaviviruses explore multiple conformations via the structural dynamics of viral envelope proteins in the virion. This adds complexity to the antigenic surface of the virion, as virus "breathing" varies the epitopes available for antibody (Ab) binding. A recent study explored the structural basis for genotypic differences in the neutralization potency of a DENV-1 specific mAb (Austin et al., PLOS Pathogens, 2012). mAb E111 binds a poorly exposed domain III epitope on the envelope (E) protein and neutralizes strain 16007 >4000x better than the related strain WP. This result could not be explained by differences in the affinity of E111 for each of these strains. Instead, the ensemble of structures sampled by these two viruses was hypothesized to differ. To further investigate differences in the "breathing" of these two DENV strains, reciprocal WP and 16007 mutants were generated that individually expressed all 13 amino acid differences in the E protein. Using DENV reporter virus particles, these variants were tested for their stability in solution (intrinsic decay) and neutralization sensitivity to a panel of mAbs. Strikingly, differences in the behavior of WP and 16007 mapped to E protein residue 204, located well outside the E111 epitope in domain III. The intrinsic decay rate of WP was ~2x greater than 16007; this difference could be reversed in a reciprocal fashion in the presence of this 204 substitution. The large difference in neutralization sensitivity of these two strains to mAb E111, and a related domain III mAb E98, was significantly modulated by the same residue. Our results demonstrate that neutralization susceptibility can be altered in an epitope-independent manner by subtle mutations (K→R) that alter the overall structural ensemble. That different conformational ensembles of flaviviruses can affect the landscape available for Ab binding, as well as virus stability, has important implications for vaccine development and antibody mapping studies.

1399

THE ANTIGENIC DETERMINANTS OF SEROTYPE SPECIFICITY FOLLOWING NATURAL DENV-3 AND DENV-4 INFECTION

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Dengue virus (DENV) occurs as four serotypes, DENV-1 through DENV-4, and is the most important arthropod-borne viral disease of humans worldwide. Infection with one serotype confers protective immunity to that serotype but not the remaining serotypes-rather, subsequent infection with a heterotypic serotype is associated with an increased risk of severe disease. Despite its worldwide importance, the antigenic determinants on each DENV serotype targeted by protective human antibodies have not been well defined. This knowledge gap has significantly hampering vaccine development. We have recently described the hinge region between domain I and II of the dengue E protein as a target of some human monoclonal antibodies that neutralize DENVs. In the current study we have transplanted the EDI-II hinge between serotypes to determine if this region is the main target of serotype specific neutralizing Abs that develop following primary DENV infections. Transplantation of EDI-II hinge from DENV-4 into DENV-3 leads to a near complete loss of DENV-3/4ic neutralization by monotypic DENV-3 human immune sera and the near complete gain of sensitivity to neutralization by monotypic DENV-4 sera. These results have important implications for vaccine design strategies as well as basic studies of dengue virus biology, immunity, and immunopathogenesis.

1400

STUDY OF EPITOPES, AVIDITY AND NEUTRALIZING POTENCY OF FLAVIVIRUS GROUP-REACTIVE HUMAN MONOCLONAL ANTIBODIES DERIVED FROM SECONDARY DENGUE VIRUS INFECTION

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The envelope (E) protein of dengue virus (DENV) is the major target of neutralizing antibodies (Abs) and vaccine development. Previous studies of polyclonal human sera after DENV infection revealed that a significant proportion of anti-E Abs were cross-reactive to all four DENV serotypes and to one or more other flaviviruses, known as group-reactive (GR). Studies of mouse anti-E monoclonal antibodies (mAbs) reported that GR mAbs were weakly or non-neutralizing compared with type-specific mAbs; GR response was thus regarded as useless for vaccine strategy. The epitopes of human GR mAbs remain largely unknown. In this study, we investigated the epitopes, binding avidity and neutralization potency of 32 human GR anti-E mAbs. The epitopes involved either fusion loop (FL) residues in E protein domain II only or both FL and bc loop residues in domain II; these residues were highly conserved by different flaviviruses and absolutely conserved by the four DENV serotypes. The neutralization potency and binding avidity of GR mAbs derived from secondary DENV infection were stronger than those derived from primary infection. Analysis of repertoire of anti-E mAbs dereived from patients with primary DENV infection revealed that the majority were GR, low avidity and weakly neutralizing, whereas those from secondary DENV infection were primarily GR, high avidity and potent neutralizing. Our observations suggest the weakly neutralizing GR anti-E Abs generated from primary DENV infection become potent neutralizing against four serotypes after secondary infection. The finding that dengue immune status of host affects the quality of cross-reactive Abs generated may have implications for different strategies of DENV vaccine.

1401

QUANTIFICATION OF TYPE I INTERFERON SIGNALING IN CELLS INFECTED WITH FIELD STRAINS OF DENGUE VIRUS

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Dengue virus (DENV), as well as other flaviviruses, circumvent the anti-viral response induced by type I interferon (IFN-α/β) by blocking key players of the JAK/STAT pathway. The relevance of the IFN-α/β system with regards to pathogenic outcomes has been highlighted in gene expression studies of DENV-infected patients showing suppression of interferon stimulated genes in patients with severe dengue. Some studies have suggested that not all DENV or flaviviruses are capable of blocking IFN-α/β signaling. Furthermore, studies of JEV and WNV have suggested a correlation between disease severity and the ability to inhibit IFN-α/β signaling. We have compared the relative inhibition of IFN-α/β signaling by DENVs using a new method that combines flow cytometry and a four-parameter logistic regression model. Clinical isolates from all DENV serotypes and isolates encompassing the five DENV-2 genotypes (Asian, American, Asian/American, Cosmopolitan, and Sylvatic) were selected and analyzed for their IFN-α/β blocking ability. We used the prototypical DENV-2 strain 16681 as a reference strain to normalize the quantitation of IFN-α/β inhibition by DENVs. The inhibitory effect of other DENVs on STAT1 phosphorylation was compared to 16681 using calculations obtained from a four-parameter logistic (4PL) model. All of the DENV serotypes and DENV-2 genotypes analyzed were able to inhibit STAT1 phosphorylation. Modest differences were observed in DENV-3 and DENV-2 sylvatic viruses. We were unable to correlate the relative strength of DENVs to inhibit IFN-α/β signaling with their plaque size or replication capacity. The quantitative method we developed allows us to determine the relative IFN-α/β blocking ability among DENV strains. Contrary to previously published studies of DENV and other flaviviruses, the majority of DENV strains analyzed in this study show a highly conserved ability to inhibit IFN-α/β signaling with a similar magnitude to that observed with DENV strain 16681. Therefore, the probability of correlating pathogenic outcomes in dengue to IFN-α/β signaling inhibition appears to be slim.

1402

EXPLORING THE MECHANISM AND SIGNIFICANCE OF CELL TYPE-DEPENDENT NEUTRALIZATION OF FLAVIVIRUSES

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Flaviviruses assemble at and bud into the endoplasmic reticulum as immature virions containing two glycoproteins, envelope (E) and premembrane protein (prM), arranged in heterotrimeric spikes. Virion maturation involves the cleavage of prM by the cellular serine protease furin. While this cleavage is required for infectivity, it may be inefficient, leading to release of partially mature virions with uncleaved prM. The maturation state of the virion has been shown previously to impact neutralization via changes in epitope accessibility. In this study, we explored the possibility that virion maturation may contribute to cell type-dependent neutralization patterns observed with many monoclonal antibodies (mAbs). We characterized the neutralization activity of a panel of mAbs using multiple target cell types. Several mAbs were significantly

less potent when assayed on Vero or BHK cells, as compared to Raji cells expressing DC-SIGNR; antibody dose-response curves revealed a resistant fraction reminiscent of our studies with antibodies sensitive to the maturation state of the virion. Our data revealed that the apparent inability of antibodies to neutralize WNV when assayed on Vero or BHK was due to the differential impact of uncleaved prM on the specific infectivity of the virus on a given cell type rather than the capacity of the antibody to block infection per se. prM+ viruses are under-represented in neutralization studies using the Vero/BHK cellular substrates typically used in neutralization assays. Analysis of sera from recipients of two live-attenuated dengue virus vaccines revealed a strong correlation between the impact of virion maturation and cell-type dependent patterns of neutralization. The neutralizing potential of cross-reactive responses may be significantly under-represented by the "gold-standard" plaque reduction neutralization test that employs Vero cells.

1403

COHERENT IMMUNE REPERTOIRE SIGNATURES IN HUMAN DENGUE

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Dengue, caused by dengue virus (DENV), is the most prevalent mosquito-transmitted viral disease of humans. The lack of early prognostics, licensed vaccines and therapeutics contributes to tremendous disease burden in endemic areas. In this study, we employed high-throughput sequencing methodologies to capture B cell-associated rearranged immunoglobulin variable heavy chain (V_H) signatures in peripheral blood mononuclear cells (PBMCs) from individuals enrolled in two ongoing dengue studies in Nicaragua. PBMCs were sampled from 44 dengue patients during acute symptomatic dengue (2-5 days post-symptom onset, "dpo"), convalescence (7-47 dpo) and post-convalescence (~180 dpo); 8 individuals with non-dengue febrile illness during the acute phase of disease; and 8 healthy individuals with no prior history of dengue. In addition, an independent set of 16 individuals with symptomatic dengue was sampled during the acute phase of illness. Analysis of V_H sequences from total PBMCs revealed clonal B cell expansion in acute dengue that was greater in secondary than primary DENV infections and not observed in convalescent and post-convalescent samples. We also identified convergent DENV-specific antibody sequences within the hypervariable complementarity determining region 3 (CDR3) that define prevalent and specific indicators of DENV infection; these CDR3 signatures were present in acute symptomatic dengue, significantly reduced after clearance of DENV infection, and not observed in non-dengue samples. The convergent CDR3 regions originated from distinct V_H sequences that were encoded by multiple V genes and were derived from B cell populations that had undergone affinity maturation and accumulated somatic mutations in response to DENV infection. These CDR3 regions and their associated CDR2 and CDR1 sequences have similar amino acid physiochemical profiles that uniquely position them as immune repertoire indicators in human dengue. This is the first report of convergent antibody sequences elicited in response to dengue, and, notably, in response to any natural infection in humans. Similar approaches using samples from individuals infected with different DENV serotypes (and genotypes) could facilitate identification of serotype-specific (and possibly genotype-specific) immune repertoire signatures. Future efforts will also be directed at assessing the antigen specificities of these convergent antibodies.

1404

INCIDENCE OF ACUTE GASTROENTERITIS-ASSOCIATED MORTALITY AMONG CHILDREN UNDER FIVE YEARS OF AGE IN BANGLADESH, 2010-12

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In Bangladesh, diarrhea-related deaths are common among children <5 years. The objective of this study was to estimate acute gastroenteritis-related mortality among children <5 years. We randomly selected 20 unions, the smallest administrative unit in Bangladesh, from the catchment areas of 11 tertiary hospitals from July to December 2012. We used social-networking to identify children aged <5 years who died in the previous two years in the targeted communities. Family members who had taken care of children during the illness preceding death were interviewed about disease symptoms and the types of healthcare sought during the illness. We classified a death as being associated with acute diarrhea if caregivers reported sudden onset of loose, watery stool ≥3 times a day within 14 days of death; we classified a death as related to acute abdomen if caregivers reported sudden onset of abdominal pain without diarrhea within a week of death. We calculated the incidence of acute gastroenteritis-related mortality by dividing the number of deaths associated with acute diarrhea or acute abdomen by the age-specific census population of the study unions. We identified 312 deaths among children <5 years; 41 (13%) following acute diarrhea and 12 (4%) following acute abdomen. Of the 53 children who died with acute gastroenteritis, 43 (81%) were aged <2 years and 26 (49%) were male. The annual incidence of acute gastroenteritis-related deaths per 10,000 children <5 years was 3.7 (95% CI 2.7-4.8), and 3.0 (95% CI 2.2-4.0) for acute diarrhea-related death. Twenty eight of 53 (53%) children died in November to February during 2010-12. Thirty five of 53 (66%) received treatment from certified physicians or hospitals within four days of illness onset and 28 of 53 (53%) died at home. The burden of acute gastroenteritis-associated mortality was highest among children <2 years. The months in which deaths peaked correspond with seasonal peaks of rotavirus circulation in Bangladesh, suggesting that this pathogen may contribute importantly to child deaths. The planned introduction of rotavirus vaccine could substantially reduce childhood mortality in Bangladesh. Many children did not seek care from trained providers, and more than half died even after seeking qualified care, suggesting that quality care may not have been accessible to these children. Improving access to prompt management of childhood gastroenteritis could save lives.

1405

HOME-BASED DIARRHEA CASE MANAGEMENT AND THE RISK OF ALL-CAUSE MORTALITY IN THE KENYA GLOBAL ENTERICS MULTICENTER STUDY (GEMS) SITE

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Modeling studies suggest that oral rehydration solution (ORS) may prevent most diarrheal deaths if universal coverage is achieved. Globally, only one-third of children with diarrhea receive ORS, the form of rehydration for diarrhea recommended in the Integrated Management of Childhood Illness guidelines. Achieving 100% ORS use will require substantial financial input and behavior change efforts. To strengthen the case for promoting appropriate diarrhea case management at home, we evaluated whether home-based diarrhea treatment methods reduced the risk of death in children with moderate to severe diarrhea (MSD). At the GEMS Kenya field site, we enrolled children <59 months old with MSD. Health workers asked caretakers about diarrhea case management at home during the time preceding enrollment. Survival status was determined at follow-up 50-90 days after enrollment. We calculated risk ratios (RR) and 95% confidence intervals (CI) to describe associations between the following and death: oral rehydration therapy (ORT) consisting of ORS, recommended home-fluids, or increasing fluids; ORS with or without other fluids; and continued feeding (CF). During 2008-11, 1,476 MSD cases were enrolled; 1,419 (96%) were followed up. At home, 65% of children received one or more forms of ORT, 14% received ORS with or without other fluids, and 19% were offered continued feeding. Between enrollment and follow-up, 52 deaths occurred (case fatality rate 3.7%), 35% of which reportedly occurred < 7 days after enrollment. Children with MSD who later died were more likely to present with slow return skin pinch and restless/irritable mental status at enrollment, and to require intravenous hydration and hospitalization during treatment than those who survived (P<.0001 for all). Home diarrhea case management strategies were not significantly associated with mortality (ORT: RR=0.92 [CI 0.53-1.61], ORS: RR=1.65 [CI 0.87-3.16], and CF: RR=0.45 [CI 0.18-1.12]. In Kenya, children with MSD experienced a high case fatality rate, but few were offered ORS and continued feeding at home. Clinical signs suggesting dehydration were associated with mortality. A small number of deaths and low rates of ORS use and CF reduced our ability to identify protective effects of ORT, ORS, and CF among children with moderate-to-severe diarrhea.

1406

ASSOCIATION BETWEEN ENTEROPATHOGENS, DIARRHEA AND GROWTH IN THE MAL-ED COHORT

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The pathogenicity of enteric infections is typically defined by their association with diarrhea. However, enteric infection has also been linked to stunted growth and decreased cognitive development. It is not clear whether the pathogens most clearly associated with diarrhea are equally important for these long-term outcomes. We performed an interim analysis of the association between enteropathogens and both diarrhea and poor linear growth in the multisite MAL-ED cohort study. To estimate pathogen-specific burdens of diarrhea, we calculated the population attributable fraction (AF) of diarrhea for each pathogen that had a statistically significant association with diarrhea. We constructed models using nine-month linear growth intervals to estimate the association between enteropathogen infections and linear growth. We then developed pathogen-specific models to determine the relative effect of symptomatic and asymptomatic infections on growth. In the first year of life, the top three causes of community diarrhea were rotavirus (aggregated AF 4%), astrovirus (3%), and enterotoxigenic *E. coli* (ETEC; 3%). *Campylobacter sp.* had the highest burden of diarrhea at three sites (Brazil, Peru, and South Africa) but was not significantly associated with diarrhea at the other sites. In the second year of life, rotavirus (aggregated AF 7%), *Shigella* (4%), astrovirus (4%), ETEC (4%), and *Cryptosporidium* (4%) had the highest burdens of diarrhea. The pathogens associated with poor linear growth were *Campylobacter* (aggregated average height loss of 0.06cm per nine-month interval), *Giardia* (0.03cm) and *Cryptosporidium* (0.01cm). In the pathogen-specific models, most of this growth burden was mediated by asymptomatic infection. Our preliminary findings suggest that the pathogens associated with growth shortfalls are different than those associated with diarrhea. These findings have substantial implications for prioritizing interventions designed to address both the mortality and morbidity associated with these infections in children in low-income countries.

1407

ENTEROAGGREGATIVE *ESCHERICHIA COLI* ASSOCIATED WITH MALNOURISHED CHILDREN IN THE MAL-ED CASE-CONTROL STUDY IN FORTALEZA, CEARÁ, BRAZIL

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Enteroaggregative *Escherichia coli* (EAEC) is an important enteric pathogen worldwide, but the disease pathophysiology remains obscure. This case-control study aimed to describe the prevalence of EAEC and potential associations of its VRGs with risk or protection to malnourished (moderate to severely underweight children, determined by weight for age Z score (WAZ) <-2) and nourished (age, sex, and neighborhood matched controls

presenting WAZ >-1) Brazilian children. Stool samples were collected from 259 children, 132 cases and 127 controls, aged 6 to 24 months that visited the specialized clinic in infant malnutrition (IPREDE) in Fortaleza, Ceará, Brazil, from Aug/2010 to Jul/2012. The specimens were cultured for *E. coli*, which was screened using microbiological standard methods. *E. coli* strains were tested for EAEC by polymerase chain reaction (PCR). For each sample, a pool of up to 5 single colonies from original MacConkey plates were examined for the EAEC diagnostic genes (aaiC and aatA). Some positive strains were individually analyzed by multiplex PCR to identify 18 VRGs. EAEC (aaiC+ and aatA+) was significantly found in 34% (45/132) of cases and 21% (27/127) of controls (P=0.021). Among these positive strains, 62 EAEC isolates obtained from 19 children, 15 cases and 4 controls, were further investigated by multiplex PCR. All EAEC strains carried at least three of the 18 assayed VRGs. The transcriptional activator aggR was the most common (98.54%), followed by genes encoding the mucinase pic (95.2%) and the hypothetical cryptic protein orf3 (93.5%). Heat-stable toxin EAST-1 and hypothetical hemolysin orf61 genes were strongly associated with cases among the EAEC strains tested (P=0.0003, OR=31.47, 95%CI=1.77-557.8; and P=0.006, OR=8.25, 95%CI=1.89-35.98, respectively). In addition, genes encoding the toxin sat and protease sepA were significantly more detected in controls compared to malnourished children (P=0.0001, OR=0.03, 95%CI=0.002-0.48; and P=0.023, OR=0.21, 95%CI=0.06-0.73, respectively). These data confirm a high prevalence of EAEC strains in the studied population and the higher association with malnourished children. Our plans include completing the EAEC VRGs characterization in all isolates to determine the importance of a combination of these VRGs potential associated with its pathogenesis.

1408

SOCIO-ECONOMIC, MOTHER CHARACTERISTICS AND CHILD CARE RISK FACTORS ASSOCIATED WITH MALNOURISHED CHILDREN IN THE MAL-ED CASE-CONTROL STUDY IN FORTALEZA, CEARÁ, BRAZIL

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In Fortaleza, located in the poorest region of Brazil, there is a large socioeconomic and cultural disparity that maybe influences the prevalence of malnutrition and its serious consequences for growth and cognitive development in children. The aim of this study was to evaluate the risk factors related to the child and the mother, as well as environmental and socioeconomic factors associated with the development of malnutrition in children in this population. A total of 345 children aged 6 to 24 months that visited the specialized clinic in infant malnutrition (IPREDE) in Fortaleza, Ceará, Brazil, from Aug/2010 to Mar/2013 were enrolled in this study. Cases (N=165) were defined as children with weight-for-age (WAZ) z-score less than -2 and control (N = 180) healthy children with WAZ more than -1. The children were monitored for anthropometric parameters and morbidity at baseline and at quarterly visits for a year. Specific questionnaires were developed and used for data acquisition on risk factors. The children in both groups did not differ by gender and age. The controls had a better birth weight, length and head circumference compared to cases (p<0.01). In relation to breastfeeding the controls showed a significant increase in the percentage in the first 24 hours (p<0.01) as well use more colostrum (OR=0.24; CI95%:0.11-0.55; p<0.01) than the cases and maintained breastfeeding longer than cases (OR=0.60; CI95%:0.38-0.94; p=0.01). The pattern of introducing liquids in the first three months of life was significantly more favorable for the controls (p<0.01). Regarding to mother´s educational level there was a significant increase in school years in controls compared with cases (OR=0.38; CI95%:0.17-0.87; p=0.01). The controls had a higher percentage in the use of piped water in the house compared to the cases (OR=2.16; CI95%:0.93-5.12; p=0.05). A multivariate hierarchical analysis is needed to determine the influence of variables on the combined outcomes and it is in progress now. In conclusion, the data showed that the weight, length and head circumference at birth, delayed initiation of breastfeeding after birth, deficit in the colostrum intake, low maternal education and poor quality of sanitation are risk factors associated with infant malnutrition in this population.

1409

CATCH-UP GROWTH OCCURS WHEN DIARRHEA BURDEN IS LOW IN EARLY CHILDHOOD

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Diarrhea and linear growth faltering continue to burden low-income countries and are among the most important causes of illness and death during early childhood. Diarrhea is thought to adversely affect linear growth, but catch-up growth can occur if no further insults are experienced. We sought to characterize catch-up growth in relation to frequency of diarrhea in a multi-site setting. Using longitudinal anthropometry and diarrheal surveillance from seven cohort studies in four countries, we examined the relationship between diarrhea prevalence and length v*eloci*ty in 3- to 6-month periods using linear mixed effect models. The v*eloci*ty during each period was calculated from the models as a function of age using linear splines. We incorporated the longitudinal prevalence of diarrhea in both current and previous periods into the model. Diarrhea during the current period was associated with slower growth in all age groups except for 0-3 months. Faster (catch-up) growth in length was observed in children with no diarrhea in the current period following a period in which diarrhea was experienced (6.01-12 month age group: 0.03 mm per month for each percent diarrhea prevalence in the previous period (95% CI: 0.009, 0.05); 12.01-18: 0.03 (0.02, 0.05); 18.01-24: 0.03 (0.002, 0.06)). Similar results were observed when weight was the outcome variable. When diarrhea episodes are followed by diarrhea-free periods in the first two years of life, catch-up growth is observed. Catch-up growth can allow children to regain their original trajectories given no or reduced diarrhea burden in subsequent periods. Diarrhea burdens are high throughout the first two years of life in developing countries, therefore reducing the likelihood of catch-up growth. Extending diarrhea-free periods may result in improved catch-up growth and a lower level of stunting. Diarrhea-free periods can be attained through expanded implementation of well-documented interventions (e.g., rotavirus vaccine, breastfeeding, zinc supplementation, and improved water and sanitation).

1410

PREVALENCE OF NON-*JEJUNI/COLI CAMPYLOBACTER* SPECIES DETECTED BY ENZYME IMMUNOASSAY AND CULTURE IN THE MAL-ED COHORT STUDY

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Methods for detection of *Campylobacter* species include culture, enzyme immunoassay (EIA) and PCR. A large discrepancy in detection by culture and EIA was noted in diarrheal and asymptomatic surveillance samples tested by both methods in the MAL-ED cohort study: 4.0% vs. 32.4% respectively in Bangladesh and 4.4% vs. 20.0% in Peru. To better understand this discrepancy, we randomly selected a total of 436 samples comprised of diarrheal cases and matched controls from children 0-12 months of age from Tanzania, Bangladesh, and Peru. According to the study protocol, all samples had previously been tested by the ProSpecT *Campylobacter* ELISA as well as by selective culture in Bangladesh and Peru. Additionally, we tested all samples with a duplex PCR assay for *C. jejuni/coli* (cadF) and *C.* species (16S rRNA). 71.6% of EIA positive samples were positive for cadF and 100% were positive for *Campylobacter* 16S rRNA, suggesting that EIA positivity was associated with non-jeuni/*coli Campylobacter* species. Next, we used 16S rRNA-based primers to sequence 60 EIA-positive samples for which the 16S rRNA quantification cycle (Cq) was at least 10 cycles lower than the cadF Cq. A sequence was successfully obtained for 50 of the samples, which most closely matched known 16S rRNA from *C. hyointestinalis* subsp. *lawsonni* (48%), *C. troglodytis* (30%), *C. jejuni/coli* (16%), and *C. upsaliensis* (6%). Of these, 7 were positive by selective culture, of which 4 were *C. hyointestinalis* subs *lawsonni*, 2 were *C. troglodytis*, and one *C. jejuni/coli. C. hyointestinalis* was the most frequently matched species in Tanzania and Peru, and *C. troglodytis* was the most frequently matched species in Bangladesh. Eight 16S positive/cadF positive samples with a less than 10x discrepancy in 16S and cadF burden were also sequenced, which most closely matched *C. jejuni/coli* (87.5%) and *C. troglodytis* (12.5%). PCR reveals a high burden of non-*jejuni/coli Campylobacter* in infants in these settings, some of which is detected by enzyme immunoassay and culture. *C. hyointestinalis* subsp. *lawsonni* is of porcine origin while the reservoir for *C. troglodytis* is not clearly established. We would estimate that approximately 10-20% of EIA positive samples from this age group from these sites represent non-*jejuni/coli Campylobacter* species, the clinical importance of which is not known.

1411

COMPARISON BETWEEN POST-TREATMENT REACTIONS AFTER DEC OR IVERMECTIN IN SUBJECTS WITH LOIASIS

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Diethylcarbamazine (DEC) treatment of loiasis is complicated by severe adverse reactions that are related to the number of circulating microfilariae (MF). The cause of these reactions in unknown, but they are accompanied by a dramatic increase in IL-5 and absolute eosinophil count (AEC). Clinically similar reactions have been seen following mass drug administration of ivermectin (IVM) for control of onchocerciasis in *Loa*-endemic areas and impact the success of filariasis control programs. To directly compare post-treatment responses following DEC and IVM, we randomized 12 subjects with loiasis and <2000 MF/mL blood to receive single-dose DEC (8 mg/kg) or IVM (200 mcg/kg). Adverse events (AE), AEC and MF counts were assessed at baseline, 4, 8 and 24 hours, and 2, 3, 5, 7, 9, and 14 days. Serum was stored at all time points for additional analyses. Baseline characteristics were comparable between the two treatment groups. All study subjects experienced mild to moderately severe AEs in the first 3 days post-treatment that were similar in character and frequency in the two groups. In the DEC group, AEC decreased from baseline levels within 24 hours in all subjects (from GM 3269/μL to 1139/μL, p=.03). This was followed by a slow rise in AEC, peaking between days 2 and 9. In contrast, all subjects in the IVM group experienced a transient increase in AEC during the first 24 hours (GM 1761/μL to 4081/μL, p=.03) with return to baseline levels by day 2. MF counts decreased dramatically in all subjects by 24 hours post-therapy (GM 1074 to 0 MF/ml in the DEC group, p =.03, and 355 to 32 MF/ml in the IVM group, p=.03), although the proportion of subjects with measurable MF counts was greater in the IVM group at all time points (p<0.05 at days 1, 3 and 5). These data suggest that DEC and IVM have differing effects on microfilarial clearance and post-treatment eosinophilia. This may have important implications with respect to interventions to prevent post-treatment reactions.

1412

COMPARISON OF THE IMMUNE RESPONSE PROFILE IN SUBJECTS WITH *LOA LOA* INFECTION AFTER A SINGLE-DOSE OF DIETHYLCARBAMAZINE OR IVERMECTIN

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Post-treatment reactions can occur in patients with loiasis following administration of either diethylcarbamazine (DEC) or ivermectin (IVM) and are believed to be due to host immune responses to dying microfilariae (MF). Although a dramatic increase in IL-5 driven eosinophilia has been described post-DEC treatment of loiasis, little is known about immune responses post-IVM. To compare the immune responses following administration of these two drugs, 12 subjects with loiasis and ≤2000 MF/mL blood were randomized to receive a single oral dose of IVM (200 mcg/kg) or DEC (8 mg/kg). Complete blood counts were performed and serum collected for mediator analysis at baseline, 4, 8 and 24 hours, 2, 3, 7 and 9 days post-treatment. Whole blood flow cytometry was performed at baseline, 1 and 3 days post-treatment to assess T cell and eosinophil activation. In the DEC group, the absolute eosinophil count (AEC) decreased from baseline levels at 8 hours in all subjects; whereas, all subjects in the IVM group experienced a transient increase in AEC during the same time frame. The absolute neutrophil count increased at 8 hours post-treatment in all subjects, regardless of treatment group. Eosinophil surface expression of CD69 increased in 11/12 subjects on day 1 post-treatment. In contrast, eosinophil surface expression of CD25 increased by a median of 96% in the subjects who received DEC, but decreased by a median of 60% in the subjects who received IVM. Similar discordance was seen with respect to CD25 expression on CD4+ T cells, with a 19% increase in the DEC group and a 46% decrease in the IVM group. Serum

IL-5 levels rose significantly post-treatment in all subjects, but peaked earlier in subjects who received DEC compared to those who received IVM (8 hours vs. 2 days). Serum IL-10 and MCP-1 levels increased post-treatment only in the DEC group. The observed differences in immunologic profiles of subjects with loiasis who received DEC as compared to those who received IVM suggest that these two drugs may exert their microfilaricidal effects through different mechanisms.

1413

IS *ONCHOCERCA VOLVULUS* SUBOPTIMAL RESPONSE TO IVERMECTIN A RESULT OF SELECTION UNDER IVERMECTIN PRESSURE? INSIGHTS FROM A STUDY COMPARING IVERMECTIN AND MOXIDECTIN IN AREAS WITHOUT PRIOR IVERMECTIN MASS TREATMENT

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Control and progress towards elimination of onchocerciasis in Africa currently rely on annual ivermectin (IVM) mass treatment (CDTI). Concern has been raised about longterm CDTI selecting for parasites with 'suboptimal response', i.e. higher skin microfilaria (mf) levels than considered 'adequate response', threatening control objectives. We analysed data from a study in Ghana, Liberia and DRC in areas without CDTI for indication of 'suboptimal' response. IVM and moxidectin (moxi) had been given to 494 and 978, respectively, males and females ≥12 years with ≥10 mf/mg skin. For the 97.2% of IVM treated and the 96.6% of moxi treated with 12 months follow up, baseline levels were 41.1±31 and 39.1±30.9, respectively (mean±SD mf/mg). Ivermectin treated: 1, 6, and 12 months post dose, mf levels were >17% of baseline in 10.2%, 14.1% and 46%, and >40% of baseline in 4.6%, 3.5% and 18.3% of subjects. Maximum levels were 150%, 159% and 375% of baseline, respectively. The % of IVM treated with undetectable levels was 42.7%, 11.3% and 5.2% at 1, 6 and 12 months. Moxidectin treated: 1, 6, and 12 months post-dose, mf levels were >17% of baseline in 0%, 0% and 4.8% and >40% of baseline in 0%, 0%, and 1.1% of subjects. Maximum levels were 8.1%, 8.5% and 58.5 % of baseline, respectively. The % of moxi treated with undetectable levels was 83.2%, 91.6% and 46.5% at 1, 6 and 12 months. The data did not indicate site or pre-dose level dependency. While the higher efficacy of moxi relative to IVM ($p<0.0001$ for all endpoints tested) shows that IVM efficacy is not optimal, comparison of the data of IVM treated with criteria and analyses in the literature shows that significant percentages of 'suboptimal responders' to ivermectin are present in populations which have not been under IVM selection pressure. This suggests that the natural variability of response to IVM is larger than commonly assumed, which needs to be taken into account during the design and analysis of studies on the origin, frequency and potential impact of 'suboptimal' IVM responders.

1414

THE DEVELOPMENT OF THE LYMPHATIC FILARIASIS QUALITY OF LIFE TOOL BANGLADESH (LF-QOL BANGLADESH)

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Lymphatic Filariasis (LF) is the world's leading cause of physical disability. Despite this, little is known about LF-disability across the stages of disease progression/manifestation, gender, age and socio-economic groups. A lack of quality data on LF-disability impact makes it difficult to develop evidence based interventions targeted to key areas of need and disease stages. A review of tools currently used in the field found that they demonstrably fail to measure the majority of known impacts of LF-disability and are culturally and linguistically inappropriate for LF-endemic populations. A Lymphatic Filariasis Quality of Life Tool was developed through a multi-staged mixed methods research process including a review of known impacts of LF-disability, in-country focus groups, cross-cultural testing and refinement and reliability studies. The final tool, the LF-QOL Bangladesh, is a 72 item, four point response format tool which measures LF-disability experience across four domains: daily activity and participation, body functions, environmental factors (community supports and barriers) and psychological impacts. In-depth in-country cognitive interviewing refined and confirmed the cultural and linguistic validity of the tool. Reliability studies found the overall internal consistency (0.917) and Corrected Item-Total correlation scores (0.91-0.926) to be excellent. The results have implications for disability measurement broadly across neglected tropical diseases (NTDs) and for the development of disability measures for intervention planning and outcome measurement.

1415

CAPACITY STRENGTHENING FOR LYMPHATIC FILARIASIS MORBIDLY MANAGEMENT: EXPERIENCE FROM PANGANI HYDROCECTOMY CAMP

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The Pangani hydrocelectomy Camp was the first ever organised by the National Lymphatic Filariasis Programme in Tanzania in consultation with Department of surgery Muhimbili University of Health and Allied Sciences (MUHAS) and West African hydrocectomy programme. The Funding for this Camp was through The President Kikwete LF Fund. Village Health Workers (VHW) registered over 400 Patients during MDA, which was followed up by screening by the surgeons at Pangani District hospital and final confirmation from specialist surgeons from MUHAS and PAUSA. As well as the standard clinical examination patients were also examined for presence of microfilaria and Circulating Filarial Antigen (CFA). The camp was organised such that specialist surgeons from tertiary hospitals trained District surgeons who then worked together to carry out the surgeries. A manual from the West African hydrocelectomy Programme was reviewed and adapted for use during this camp. The focus was in the use of the Excision technique, which required the excision of the tunica *vaginalis* to ensure no recurrence. A total of 202 patients with an age range was between 9 and 86 years, were operated on in the 10 days. Out of the 200 patients 101(50.0%) had bilateral 20(10.0%)had hydroceles with hernia

and 5(2.5%)had hydrocele with testicular complications like atrophy, tumour and necrosis. Hydrocele fluid was collected for biochemical analysis and tissue sections of the Tunica *vaginalis* were preserved in formalin for histopathology. The Surgeons trained village health workers who looked after the patients post surgery in the village. A special algorithm on wound care management and danger signs was provided to all VHW and findings recorded in daily diaries, which were reviewed after 7 days. The experience indicated that large-scale camps are useful especially when they involve local personnel at district level. It also showed that Village Health workers could support postoperative care and hence the involvement of Village Health Workers in morbidity management is crucial. The camp was also a great advocacy activity inspiring more men to register for hydrocelectomies.

1416

COMPLIANCE TO LYMPHEDEMA MANAGEMENT TECHNIQUES AND ITS IMPACT ON THE RATE OF ADENOLYMPHANGITIS (ADLA) EPISODES IN KHURDA DISTRICT, ORISSA STATE, INDIA

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Lymphedema management programs have been shown to decrease episodes of ADLA, but the impact of compliance with specific lymphedema management techniques has not been explored in detail. Our objectives were to determine the rate of ADLA episodes over time for patients enrolled in a community-based lymphedema management program and determine predictors of compliance to the program. A community-based lymphedema management program was implemented in Orissa State, India from 2007-2010 by the Indian non-governmental organization, Church's Auxiliary for Social Action, in consultation with the Centers for Disease Control and Prevention. Patients (n=374) were followed over 24 months. The 30-day rate of ADLA episodes decreased from 0.34 episodes per person at baseline to 0.23 episodes per person at 24 months (P=0.0043). From baseline until 24 months after the program began, the average of compliance with each separate lymphedema management technique (limb washing with soap, anti-fungal cream use, elevation of the limb, limb exercise, and use of footwear outdoors) increased from 19.3% (2.9%-41.8%) to 65.4% (34.2%-92.2%) (p<0.0001). Ordinal logistic regression models found increasing age (OR=1.02 [1.01, 1.03]), paid work (OR=1.71 [1.21, 2.41]), and use of a mosquito net (OR=1.45 [1.08, 1.95]) to be significantly associated with compliance to limb washing with soap. Increasing lymphedema stage (OR=1.16 [1.02, 1.30]), increasing age (OR=1.05 {1.04, 1.07]) and increasing number of ADLA episodes in the last 6 months (OR=1.15 [1.06, 1.25]) were associated with compliance to wearing footwear outside the home. This study demonstrates improvement in ADLA episodes within a community-based lymphedema program. In addition, it illustrates characteristics of persons who complied with lymphedema management techniques and can assist programs in targeting those who may be less compliant in lymphedema management programs.

1417

REPURPOSED LABORATORY EQUIPMENT PROVIDE A FIELD-FRIENDLY, POINT-OF-CARE METHOD FOR QUANTIFYING *LOA LOA* MICROFILARAEMIA IN ADVANCE OF "TEST AND (NOT) TREAT" STRATEGY PREVENTION OF POST-TREATMENT SERIOUS ADVERSE EVENTS

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Administration of ivermectin as part of mass drug administration (MDA) campaigns for onchocerciasis and/or lymphatic filariasis in areas co-endemic for *Loa loa* has resulted in severe post-treatment adverse events (SAEs) including encephalopathy and death. This has led to the suspension of MDA in some of these co-endemic areas of Central Africa. One simple, potential solution aimed at preventing *Loa*-associated post-treatment SAEs is to identify and exclude individuals at risk (high levels of microfilaraemia) from the MDA in a program termed 'Test and (Not) Treat" (TNT). We describe the adaptation and optimization of an existing technology for a rapid, point-of-care method for quantifying microfilariae in the blood of infected individuals. By repurposing a handheld microfluidics-based cell counter (Scepter™), we demonstrate that microfilariae can be identified and quantified using minimal volume of whole blood (20µl) after lysis with 10% saponin. A highly significant correlation (r=0.9182, p<0.0001) was observed between counts obtained by microscopy and those obtained using the Scepter™ study using 20µl of blood with microfilariae of *Brugia malayi*, *Dirofilaria immitis* or *L. loa*. Preliminary proof of concept studies in Cameroon with 20µl of *L. loa* infected human blood (n=30) and experimentally infected baboons (n=4) with a wide range of microfilaria levels demonstrated that the counts obtained by calibrated thick blood smears and those by Scepter™ were highly correlated (r=0.8504, p<0.0001), though at very low levels of microfilaria, there was a loss of sensitivity with the Scepter™. Moreover, the time from blood draw to microfilarial count for the Scepter™ was between 1-2 minutes whereas for the calibrated thick smear the time ranged between 4 hours and 2 weeks. The data suggest that we have a sensitive, rapid, point-of-care and quantitative test to identify individuals with levels of *L. loa* microfilariae that put them at risk for SAEs. In addition, it requires minimal blood volumes, is highly portable, independent of ambient temperature and humidity and provides ease of data storage and accessibility.

1418

GLOBAL RISK MAPS OF THE *LEISHMANIA*SES

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The *Leishmania*ses are a collection of complex infections caused by *Leishmania spp.*, ranging from localised cutaneous lesions to forms with visceral complications. Annual incidence is estimated to be around 1.5 million new cases of cutaneous leishmaniasis, and 0.5 million cases of visceral leishmaniasis. The interplay between humans, the Phlebotomine sandfly vectors and reservoir hosts complicates epidemiological understanding as well as control efforts; research has therefore tended to concentrate on solving clinical and epidemiological aspects of the

disease at small spatial scales. This, combined with the comparatively little funding and research attention the leishmaniases garner, has resulted in no attempt to provide a global evidence-based risk map of these diseases. For each sub-national province, an assessment of cutaneous and visceral leishmaniasis was performed incorporating data from the WHO Expert Committee and the Global Infectious Diseases and Epidemiology Network as well as peer-reviewed disease occurrences and reported annual caseloads. These data were used to quantitatively assess certainty of the diseases' presence or absence on a continuous scale. A global database of close to 20,000 geo-positioned data points was collected from peer-reviewed literature using Web of Knowledge and PubMed searches and lab confirmed case data. Using a predictive Boosted Regression Trees modelling approach, separate continuous global risk maps for Cutaneous and Visceral Leishmaniasis were produced. We predict Leishmaniasis risk throughout Central and Southern America, as well as from the Mediterranean Basin to Western China, with other foci in Central and Southern Africa. Climatic and environmental variables were identified as important in defining this distribution. It is hoped that such a map will help inform not only future epidemiological studies but also public health policy directed towards these diseases, allowing improved targeting of specific control efforts with humans, vectors and reservoirs, as well as identify suitable areas for surveillance both active and passive.

1419

GLOBALLY FIRST TIME SYSTEMATICALLY CASE-BASED INTRODUCTION OF LLIN (LONG LASTING INSECTICIDE TREATED NET) AND ITS IMPACT IN KALA-AZAR (VISCERAL LEISHMANIASIS) ELIMINATION IN HYPER ENDEMIC SUB-DISTRICTS OF BANGLADESH

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Bangladesh along with Nepal and India is committed to Eliminate Kala-azar (Visceral Leishmaniasis) by 2015. Effective Integrated Vector Management (IVM) is one of the main strategies for Kala azar elimination and LLIN (Long Lasting Insecticide treated Net) is one of the tools for this IVM for preventing human- vector contact. The objective of systemically case based LLINs distribution is to implement the Integrated Vector Management strategy for Elimination of Visceral Leishmaniasis in Hyper Endemic sub-districts of Bangladesh and thus pave the way for elimination status by 2015 and to examine the effectiveness of LLIN tool in reducing the human- vector (Sand fly) contact, and improving awareness building in community for helping in identification of new KA /PKDL (Post Kala-azar Dermal Leishmaniasis) cases through Campaign distribution approach. Total 9494 patient both kala-azar and PKDL, registered since 2008 in 8 hyper endemic sub district of Bangladesh had received the nets. Village-unit approaches were followed. Baseline data was collected and selection of sub-district was done on endemicity criteria and cases were identified from 2008 -- August 2012. A LLIN distribution strategy and Micro plan were developed and also for BCC material (pre and post distribution). Active case search and selection of patient through field visit was done and advocacy meetings were arranged at Sub-district level to create mass awareness on LLINs. Village wise campaigns were arranged for distribution of LLINs among new and old cases. --one LLIN for each patient and extra one for his/her family members. Proper monitoring, evaluation, follow- up program was done by the field managers. Globally practiced for first time, as a tool for Integrated Vector Management (IVM) for Visceral Leishmaniasis Elimination Program, case based LLIN distribution was successfully implemented and can be practiced in other countries. To reduce the transmission of Kala azar from reservoir to Vector, LLIN distribution can play a vital role for PKDL patients. Also lessons were learned that LLIN distribution among KA/KDL cases, with prior campaign for community participation can act as Catch-up strategy for new case identification.

1420

MULTI-SCALE MIGRATION PATTERNS OF *TRIATOMA INFESTANS* IN AN URBAN ENVIRONMENT AND IMPLICATIONS FOR LONG TERM PREVENTION OF CHAGAS DISEASE

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Arequipa, with close to one million inhabitants, is Peru's second largest city. It is currently undertaking a campaign to control *Triatoma infestans*, the main vector of Chagas disease. *T, infestans* mobility has long been linked to human movement, suggesting that separate vector populations in large, interconnected urban centers such as Arequipa may behave as a large connected population. Treated households could potentially be recolonized by vectors from households that did not participate in the control campaign or by neigborhoods still awaiting treatment. Here, we develop a new spatial model-based methodology to estimate *T, infestans* migration patterns at the city-block, neighborhood, and city level. We apply this method to spatio-temporal infestation data collected during vector control activities. We estimate that an existing infested household will generate a secondary infestation in a completely susceptible population on average every 1.12 years [0.9-1.3]. We find that the rate of dispersal to neighboring city blocks is on the same order of magnitude as longer-distance dispersal and that both of these are much less common than dispersal within a city block. These estimates are compatible with previously observed auto-correlation patterns of infestation showing a strong barrier effect of streets and with genetic diversity patterns observed using microsatellite markers. The relative importance of migration to distant households suggests that propagation of infestation outside a city-block is largely due to passive transport and probably linked to human movements and is much less determined by distance than by active insect dispersal. In the context of low participation rates in the control campaign (60-85%) along with a high infestation prevalence prior to control (10-30%), we discuss the impact of migration on vector surveillance requirements over time and more generally how long-term vector control planning can be based on epidemiological data routinely collected during control efforts.

1421

SCREENING OF ANTI-INFECTIVES AGAINST *PLASMODIUM* AND KINETOPLASTIDS: A SERVICE FOR THE RESEARCH COMMUNITY

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The Anti-Infectives Screening Core, a non-profit entity created to facilitate early drug development for neglected diseases, takes advantage of the specialized facilities and expertise at NYU Parasitology for testing candidate molecules for parasitic diseases *in vitro* and *in vivo*. Currently, the core tests anti-infectives for four parasitic neglected diseases: Malaria, Chagas Disease, Human African Trypanosomiasis, and Leishmaniasis. Compounds are provided by users and shipped to the core for testing without revealing structures or any IP involvement. We have set up *in vitro* assays that determine the potency of compounds (EC50) for specific parasites: *Trypanosoma cruzi* (intra-host cell amastigotes), *Trypanosoma brucei brucei* (bloodstream forms), *Leishmania amazonensis* (promastigotes, axenic amastigotes or intra-macrophage amastigotes), *L. donovani* (promastigotes), *L. major* (promastigotes), and *Plasmodium falciparum* asexual stages, in addition to quantification of the cytotoxicity (TC50). Each assay contains negative and positive controls and each determination is performed in duplicate. In vivo assays take advantage of transgenic parasites that express luciferase, which allows rapid automated

quantification of infection. Groups of five mice are infected with any of the parasites of study: *T. cruzi, T. brucei brucei, L. amazonensis* and *P. berghei* (liver, blood stage, gametocyte or mosquito transmission). When infection has progressed, mice are imaged to quantify the luminescence signal, which is proportional to the parasite load (baseline infection level). Treatment with the test compounds begins, normally administered via i.p. injection or oral gavage. One day after the last treatment dose, mice are imaged again to determine the level of infection. Results are expressed as the ratio of infection at the end of treatment versus the base infection for each animal. Testing includes a negative control and positive control groups with a well-known drug for each disease. http://ocs.med.nyu.edu/anti-infectives-screening

1422

ESTABLISHING BIOMARKERS OF *LEISHMANIA DONOVANI* INFECTION: POTENTIAL ENDPOINTS FOR VACCINE TRIALS

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Visceral leishmaniasis in the Indian sub-continent has been targeted for elimination by 2015. The realization of this goal is dependent upon identifying early infection in asymptomatic individuals, who present no overt symptoms and are potential reservoirs for spreading infection and developing disease. We studied asymptomatic individuals in the *Leishmania donovani*-hyper endemic Mymensingh district in Bangladesh over a period of 24 months to define the natural dynamics of *Leishmania*-specific antibodies and DNA and reveal their utility as biomarkers of infection. Samples were analyzed by DAT; *L. donovani* whole cell lysate and rk39 ELISA; and quantitative PCR. Serological tests indicated the sustained presence of antibodies at study intake and at a 12-month follow up interval. By DAT, 57% tested positive at both time points while by ELISA, 82% and 89% were positive at baseline and 75% and 92% tested positive at 12 month follow-up to *L. donovani* whole cell lysate and rk39, respectively. In contrast, though 84% tested positive by PCR at baseline, only 28% remained positive at follow-up. During the course of the study, 3 of the 56 study subjects developed symptomatic VL, with each consistently testing positive for antibodies or nucleic acids. Our results reflect the transient nature of asymptomatic *L. donovani* infection in this endemic area. Used together, the presence of *Leishmania*-specific antibodies and nucleic acids can predict individuals who are at the highest risk of progression to disease and thus will gain most from intervention. Based on our results, we suggest means by which tests for *Leishmania*-specific antibodies and circulating *Leishmania* DNA can be used in active surveillance of endemic areas. We conclude that these bio markers will be valuable in identifying populations for vaccine trials as well as serve as end points to evaluate the effectiveness of the trials.

1423

NOVEL NANOTECHNOLOGY TO CONCENTRATE AND PRESERVE *TRYPANOSOMA CRUZI* ANTIGENS IN URINE FOR EARLY DIAGNOSIS OF REACTIVATION OF CHAGAS DISEASE IN PATIENTS CO-INFECTED WITH HIV VIRUS

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We developed harvesting nano-porous particles to capture, concentrate and preserve *Trypanosoma cruzi* antigens in urine of HIV/*T. cruzi* patients. Diagnosis of reactivation of chronic Chagas disease in HIV/*T. cruzi* patients is based on detection of *parasitemia* by micromethod but lacks sensitivity. Antigenuria has been shown to be correlated with *parasitemia*, but has also low sensitivity. Poly N-isopropylacrylamide (NIPAm) based particles are functionalized with chemical baits (trypan blue, TB) that capture antigens with high affinity (KD<10-12 M) within minutes, antigens captured can be eluted in a small volume yielding a concentration factor that is the ratio between the initial volume of urine and the final elution volume. In this study, model urine samples were incubated with poly(NIPAm)/TB particles. Antigens eluted from the particles were detected by Western Blot using a polyclonal antibody against *T. cruzi* H49 antigen. Nano-porous particles increased the sensitivity of antigenuria by *T. cruzi* more than 100 fold (detection limit was 0.8 ng/ml with particle treatment compared to 100 ng/ml without particle treatment). This assay was applied to a cohort of HIV/*T. cruzi* co-infected patients (N=39, 20 *T. cruzi* positive and 19 *T. cruzi* negative). Sensitivity of antigenuria in the particle-concentrated urines was 100% (2/2), 90% (9/10) and 80% (16/20) compared to micromethod, PCR and ELISA, respectively. The specificity was 100%. Positive results of antigenuria were correlated to high levels of *parasitemia* ($p<0.05$). Particle-sequestered *T. cruzi* H49 antigen was protected from enzymatic degradation by trypsin digestion and in urine over seven days at room temperature, showing that particles protected urinary antigens from degradation. Nano-porous particles effectively concentrated *T. cruzi* antigens in urine. Nanotechnology-enhanced antigenuria test could be an early predictor of reactivation and can be adapted for monitoring HIV/ *T. cruzi* co-infected patients. Particle integration in urine collection is envisioned for sample handling and shipment at room temperature.

1424

AN7973: A NOVEL OXOBOROLE FOR THE TREATMENT OF AFRICAN ANIMAL TRYPANOSOMIASIS

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African animal trypanosomiasis (AAT) is a parasitic disease caused by tsetse fly-transmitted trypanosomes, which include *Trypanosoma congolense (T.c.), T. brucei brucei (T.b.b.),* and *T. vivax*. AAT results in serious economic losses in livestock due to reduced productivity from anemia, emaciation and fever. AN7973 is a novel boron-containing molecule that demonstrates excellent potency against *T. congolense in vitro* as well as *in vivo* efficacy against *T.c* infection in mice, goats, and cattle. AN7973 has an IC_{50} of 0.057 μM and 0.098 μM against *T. c.*, and *T. b. b.*, respectively. Compound wash-out experiments demonstrate that a 10 h exposure to AN7973 results in irreversible killing at concentrations as low as 1.25 μg/mL (3.3 μM). By 24 h, 99% of the parasites were killed at concentrations of 0.15 μg/mL (0.40 μM), demonstrating the trypanocidality of AN7973. PK studies were performed in mouse, rat, dog, and cattle. Intramuscular (IM) injection of 5 mg/kg in cattle resulted in a C_{max} of 2.05 μg/mL, $AUC_{(0-24)}$ of 89.1 h*μg/mL and a terminal half life of 22.6 h. AN7973 was tested for *in vivo* efficacy in a murine model of *T.c.* infection. A single dose of 10 mg/kg showed 100% cure, 60 days after treatment. In a goat model of *T.c.* infection, 10 mg/kg by IM injection demonstrated 100% cure at Day 100. In a cattle efficacy study, using a diminazene- and isometamidium-resistant strain of *T.c.,* a single IM dose of 10 mg/kg or 2 doses of 5 mg/kg, 24 h apart, also demonstrated 100% survival at 100 days post-treatment, reflecting complete cure. The plasma concentration in the cattle efficacy study at 1 x 10 mg/kg was 1.93 μg/mL at t = 24 h, well above the concentrations necessary for cidality. The plasma exposure (AUC_{0-24h}) was determined to be 46.1 h*μg/mL. AN7973 was well tolerated in a cattle safety study during which AN7973 was dosed at 30mg/kg three times with

2 weeks separation between doses. In summary, AN7973 demonstrates excellent efficacy against *T.c.* in the target animal, and shows promise as a novel chemical entity for treatment of AAT.

1425

VILLAGE-LEVEL CHARACTERISTICS ASSOCIATED WITH SPATIAL DISTRIBUTIONS OF MALARIA-INFECTED INDIVIDUALS IN AN AREA OF SOUTHERN ZAMBIA RECEIVING MASS SCREENING AND TREATMENT

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Malaria clusters across space and time; malaria interventions may be targeted to maximize the efficiency of scarce resources. However, how to target malaria interventions toward small foci of transmission is not well understood. An ongoing mass screening and treatment (MSAT) intervention in southern Zambia has provided census data at 4 different time from Dec 2011 – Nov 2012. The difference in K function, which assesses spatial regularity or clustering compared to randomness at any distance, was used to assess the spatial distribution of malaria-infected individuals observed during each census. Individuals with a malaria infection clustered within households at all prevalence levels and month of the census. Beyond the household, individuals with malaria infections were distributed differently in space depending both on village parasite prevalence and month of the census. Because the spatial distribution of malaria-infected individuals varied by prevalence level, malaria parasite prevalence aggregated to the village level was then modeled to determine factors that may explain the differing spatial distributions. A linear mixed effects model including altitude, enhanced vegetation index, nighttime temperature, round of MSAT (categorized as round 1-4) and the topographical position index (whether a village was located in a valley, ridge, plain, or slope) accounted for 82.5% of the variation in village malaria parasite prevalence. This technique also revealed that an increase in altitude of 100m was associated with an absolute 2.5% decrease in village parasite prevalence ($p < 0.001$). An increase from 0 (dry, brown foliage) to 1 (green dense foliage) in the enhanced vegetation index was associated with an absolute 44% increase in village parasite prevalence ($p < 0.001$). A 1-degree increase in nighttime temperature was associated with an absolute 0.4% increase in village parasite prevalence ($p < 0.01$). Multiple rounds of the MSAT intervention were also associated with decreased village malaria parasite prevalence; 3 rounds were associated with an absolute 9.6% decrease (relative 38.6%) from the baseline round ($p < 0.001$). Varying spatial distributions of malaria-infected individuals appear to be driven by vector abundance and gametocyte prevalence in the population. The ability to clearly delineate village malaria prevalence may assist in developing mechanisms for focused interventions to optimize their effectiveness.

1426

RAPID SCALE-UP OF LONG-LASTING INSECTICIDAL NETS ASSOCIATED WITH A DECREASE IN SEVERE MALARIA INCIDENCE AND AN UPWARD SHIFT IN THE MEAN AGE OF SEVERE CASES AMONG CHILDREN IN LUANGWA DISTRICT ZAMBIA

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Long-lasting insecticidal nets (LLINs) have been shown to reduce malaria transmission by as much as 90% with concomitant reduction in malaria incidence and all-cause child mortality in the African Africa. Ecological data have also shown the mean age of severe malaria to shift from younger to older children as transmission decreases in these settings. In late 2005 and 2006 16,100 LLINs were distributed free of charge to all households (approximately 4,000)in the Luangwa District of Zambia, resulting in rapidly achieving high household coverage of LLINs (73%), from very low coverage prior to 2005. We assessed trends in the mean age of children under 10 years old admitted to the two hospitals serving Luangwa District from January 2003 through August 2009. A difference-in-difference analytic approach was used in a linear regression model to assess change in the mean age of reported child hospital admissions (primary outcome), categorized as malaria or non-malaria diagnosis, before and after LLIN scale-up (before and after January 2007), while controlling for hospital, malaria transmission season, and lagged monthly vegetation index and mean temperature. This approach allowed us to assess the relative change in the mean age of reported in-patient severe malaria cases compared to all hospitalized admissions due to causes other than malaria over this time period. Total reported in-patient admissions among children under 10 years old decreased from 63.8 per 1,000 in 2003 to 41.2 per 1,000 in 2009. Reported in-patient severe malaria admissions decreased from 41.7 to 21.5 per 1,000 population over this same period; severe malaria as the cause of admission decreased from 64.6% in 2003 to 51.6% in 2009. The mean age of non-malaria admissions stayed relatively constant over the observation period at 23.9 months, while the mean age of severe-malaria admissions increased from 17.1 months prior to LLIN scale-up (before 2007) to 23.5 months post LLIN scale-up (coefficient for malaria diagnosis X pre-post LLIN period interaction term = 0.47 (in years); p-value <0.001). Results suggest a decline in reported severe malaria admissions following LLIN scale-up, with a coinciding upward shift in the mean age of severe malaria admissions. These results suggest that the rapid LLIN scale-up in Luangwa district was associated with a marked decrease in malaria transmission, severe illness, and modifications in the age distribution of those afflicted.

1427

SUSTAINED DECLINING BURDEN OF MALARIA AT COMMUNITY LEVEL IN NORTHEASTERN TANZANIA

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The reported decline of malaria in most parts of Tanzania has some implication on accuracy of malaria diagnosis and management, especially following the introduction of expensive artemisinin combination therapy (ACT) with artemether/lumefantrine (ALu). Traditionally, fever has been the back-bone of malaria case management; but with declining malaria and

introduction of expensive ACTs, this approach poses a major challenge. In our previous and ongoing malaria passive case detection in 4 villages of Korogwe, northeastern Tanzania, we demonstrated that provision of early diagnosis and treatment of malaria by community owned resource persons (CORPs) using rapid diagnostic tests (RDTs) and ALu is an effective strategy for malaria control. We now provide updates on sustained impact of these interventions on malaria in communities where the transmission has significantly declined. In 2006, individuals with history of fever within 24 hours or fever (≥37.5°C) at presentation were presumptively treated with sulphadoxine/pyrimethamine. Between 2007 and 2012, individuals aged 5 years and above with positive RDTs were treated with ALu while under-fives were treated irrespective of RDT results. A total of 18,981 cases were attended and 17.2% were positive for malaria parasites by microscopy. Malaria prevalence and incidence decreased across the years, from 34.6% to <1% and 235/1000 to <8/1000 person years at risk for 2007 and 2012, respectively. The highest incidence of malaria shifted from children aged 5-9 years to individuals aged 10-19 years from 2009. Despite these changes, fever prevalence remained high at >40.0% in under-fives and >20.0% among individuals aged 5 years and above. The significant reduction in malaria prevalence and incidence observed might be attributed to different interventions including early diagnosis and prompt treatment through CORPs strategy. Studies to investigate causes of fevers other than malaria are recommended for better case management. The current remarkable and sustained decline in malaria suggests that these areas might be moving from control to pre-elimination levels.

1428

RESERVOIRS OF ASYMPTOMATIC MALARIA IN MALAWI: RESULTS OF TWO CROSS-SECTIONAL STUDIES

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Malaria surveillance in endemic countries typically focuses on young children who are at highest risk of malaria morbidity and mortality. As we develop strategies to eliminate malaria, it is critical to expand our understanding of sources of malaria transmission. The Malawi International Center for Excellence in Malaria Research conducted cross-sectional surveys in the 2012 rainy and dry seasons in three transmission settings in southern Malawi with the goal of estimating prevalence of asymptomatic malaria infection and assessing risk factors for asymptomatic *parasitemia* in each setting. Districts were selected to represent urban/low (Blantyre City), rural/high (Chikhwawa), and semi-rural/mountainous (Thyolo) malaria transmission. We randomly selected 30 households in 10 enumeration areas in each district. Demographic, malaria intervention, and current health status data were collected through household interviews; blood samples were obtained from all individuals over six months of age. Among 5099 individuals with smear results in Blantyre, Chikhwawa, and Thyolo, total parasite prevalence was 11.7%, 13.1%, 11.0% in rainy and 3.9%, 17.4%, 9.4% in dry seasons respectively. Asymptomatic *parasitemia* represented 46.2%, 41.7%, 49.3% and 76.7%, 69.5%, 79.0% of total parasite prevalence in the two seasons, respectively. In multinomial regression using aparasitemic individuals and age 6-59 months as reference groups and controlling for district, individual net use and indoor residual spraying, ages 5-15 years was strongly associated with asymptomatic *parasitemia* in the rainy season (Odds ratio (OR) = 6.7, [95% Confidence interval (CI): 3.3, 13.7]) and also in the dry season (OR = 1.5 [95% CI: 1.1, 2.2]). Age >15 years was not significantly associated with asymptomatic *parasitemia* in the rainy season but was protective (OR = 0.64, [95% CI: 0.45, 0.92]) in the dry season. In Malawi and potentially in other endemic settings, school age children represent important reservoirs of asymptomatic infection and should be targeted for interventions to interrupt transmission.

1429

MICROEPIDEMIOLOGY OF SUB-MICROSCOPIC *PLASMODIUM FALCIPARUM* INFECTION: IMPLICATIONS FOR DETECTION OF HOTSPOTS WITH IMPERFECT DIAGNOSTICS

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At the local level, malaria transmission clusters in hotspots, which may be a single household or group of households that experience higher than average exposure to infectious mosquitoes. Active case detection (ACD), often relying on rapid diagnostic tests (RDTs) for mass screen and treat campaigns, has been proposed as a method to detect and treat individuals in hotspots. Here we used data from a cross sectional survey conducted in north-western Tanzania to examine the spatial distribution of *Plasmodium falciparum* to establish whether RDTs are likely to have sufficient sensitivity to target ACD interventions aimed at reducing transmission. Dried blood spots were collected from all consenting individuals from four villages in a single ward during a survey conducted between August and November 2010. These were analyzed by PCR for the presence of *P. falciparum*, with the parasite density of positive samples being estimated by quantitative PCR. Household exposure was estimated using distance-weighted PCR prevalence of infection. Results showed that mean distance-weighted PCR prevalence per household was 34.5% (range 0 - 94.7%). Infection density was highest in children 5-10 years old and lowest in those >40 years old. Infection density was negatively associated with transmission intensity with the odds of an infection being sub-microscopic increasing with household exposure (OR 1.09 per 1% increase in exposure, p<0.001). This relationship, which is potentially explained by exposure-related immunity, suggests that RDTs and microscopy have the lowest sensitivity in transmission hotspots. Simulations of different targeted mass drug administration (tMDA) strategies showed that treating all individuals in households where RDT prevalence was above 20% increased the number of infections that would have been treated from 43% to 55%, however, 45% of infections remained untreated. Even using a single RDT positive as a trigger for household MDA resulted in around 35% of infections remaining untreated. Taken together, these results suggest that community wide MDA, instead of screen and treat strategies, may be needed to successfully treat the asymptomatic, submicroscopic parasite reservoir and reduce transmission in similar settings.

1430

OPERATIONAL APPROACHES FOR DETECTING FOCI OF MALARIA INFECTION: HOW DO SCHOOL AND HEALTH FACILITY SURVEYS COMPARE AGAINST A COMMUNITY-BASED APPROACH

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There is increasing evidence of heterogeneity manifested through presence of foci of malaria infection. Tailoring interventions to reflect this heterogeneity is likely to bring benefits in terms of their impact and cost effectiveness. For such a targeted approach to be effective in the long term, strategies are needed to enable local malaria control teams to reliably identify and target the true foci of infection in the community.

In July 2010, 4987 children were tested for malaria across 46 schools in Rachuonyo South district in western Kenya and the compounds of 4888 children (98%) were geolocated. Two surveys were conducted in 5 health facilities in the same area (Oct 2011, July 2012) with a combined total of 3034 people tested for malaria. Of the participants sampled, spatial coordinates of the compound were obtained for 30% of the participants. All participants sampled at school and health facilities were tested for malaria by rapid diagnostic test and samples from all surveys were assessed for antibody response to *Plasmodium falciparum* AMA1 and MSP1. The results were compared to foci of infection identified during a community cross-sectional survey of 17506 individuals. Preliminary results indicate that if positive for malaria by RDT or serology, participants had twice the odds of residing in foci of infection (p<0.0001). Seropositivity in schools surveys had a sensitivity of 64.1% in identifying children that reside in known foci whereas RDT results obtained during the health facility surveys were 86.8% specific in identifying children that do not reside in known foci of infection. Results indicate that school and health facility surveys may provide an alternative approach to detect foci of infection in the community. However, the definition of foci of malaria infection from both an operational and academic perspective is in need of further discussion.

1431

USING SEROLOGICAL MARKERS FOR ESTIMATING MALARIA TRANSMISSION INTENSITY AND ASSESSING INTERVENTION EFFICACY IN WESTERN KENYA

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Accurate measurement of local malaria transmission is critical for evaluating control interventions. Serological conversion rates (SCRs) have been used to estimate the force of malaria infection in populations. In low to moderate transmission settings, systematic reductions in incidence (e.g., due to effective interventions) can be measured through a single retrospective serological survey. Our objective was to validate, in a malaria hyperendemic region, the accuracy of serological markers for 1) estimating transmission intensity and 2) retrospectively detecting a decline in incidence. Asembo, western Kenya, is an area that experiences high, perennial malaria transmission. From 1997-1999, Asembo was the site of a community-wide insecticide-treated bed net (ITN) trial that reduced malaria transmission by 90%. Serological samples collected pre-ITN (1994) and post-ITN (2009) were tested by indirect ELISA for antibodies against *Plasmodium falciparum* circumsporozoite protein (CSP), merozoite surface protein-1 (MSP-1), and apical membrane antigen-1 (AMA-1). Age-specific seroprevalence data were fitted to catalytic conversion models to estimate SCRs for 1994 and 2009. Post-ITN (2009) age-seroprevalence curves were also examined for tiered trends to denote when transmission declined. Between 1994 and 2009, SCRs for CSP, MSP-1, and AMA-1 fell by 50%, 25%, and 49%, respectively. SCRs corresponded closely with entomological inoculation rates, which dropped from >100 to 10 infectious bites/person-year during this 15 year period. Post-ITN (2009) SCRs were uniform across all ages rather than tiered; older age groups born before the trial did not exhibit higher SCRs than young age groups born after. We conclude that serological markers provide reliable estimates of malaria transmission intensity near the time of sample collection. Because we were unable to pinpoint when the drop in transmission occurred, however, this model did not appear to be accurate in malaria hyperendemic areas for retrospective reconstruction of historical trends associated with control interventions.

1432

INNATE LYMPHOID CELL POPULATIONS DRIVE THE TH2 IMMUNE RESPONSE TO *LITOMOSOIDES SIGMADONTIS* IN BALB/C MICE

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A group of innate cells, termed nuocytes, multipotent progenitor (MPP) cells or innate helper cells (IHC) [collectively referred to as innate lymphoid cells (ILCs)] have been identified at barrier surfaces and are involved in propagating a Th2-type response following infection with intestinal helminths in mice. *Litomosoides sigmodontis* (Ls) is a filarial parasite of rodents that lives in the pleural cavity and develops a Th2-response at patency (when microfilariae are produced) at day 42 post infection (p.i.). To assess whether the pleural cavity also utilizes ILCs to drive a Th2 response, Balb/c mice were infected with 40 infective stage larvae (L3) of Ls and the frequencies of ILC subpopulations were determined by multiparameter flow cytometry of cells isolated from the spleen and pooled samples from the pleural cavity on days 5, 14, 36, 42, and 60 p.i. ILCs were defined as lineage-/cKit+ and were further divided by Sca1+ (MPPs), Sca1-/CD90.2+/CD44+ (IHCs) and Sca1+/CD90.2+/CD44+/ST2+ cells (nuocytes). Each of these ILC subpopulations was identified in the spleen and pleural cavity of infected and uninfected mice. Two of the 3 ILC subpopulations were significantly expanded in the spleen at day 42 p.i., compared to uninfected matched controls (nuocytes: p= 0.021, IHC: p=0.044). In the pleural cavity, there was an increased frequency of MPPs and nuocytes at day 36 and day 42 p.i. compared to controls and an increase of IHCs at day 42 p.i. The cellular infiltrate in the pleural cavity during infection showed that neutrophils (22-fold day 36, 68-fold day 42), eosinophils (23-fold day 36, 9-fold day 42) and macrophages (26-fold day 36, 6-fold day 42) increased markedly at day 36 to day 42 p.i. This increase was accompanied by an increase from baseline in the levels of IL-4 (16-fold day 36, 34-fold day 42), IL-5 (144-fold day 36, 218-fold day 42), IL-13 (167-fold day 36, 3-fold day 42) and IL-10 (1.5-fold day 36, 27-fold day 42) in the pleural lavage fluid and by increases in plasma IL-5 levels (p=0.0097 day 36, p=0.0570 day 42) and levels of IgE (p-value=0.0079 day 42) and IgG1 (p-value=0.0079 day 42) antibodies. These data confirm the induction of a Th2-dominant response both locally and systemically by Ls and suggest that ILCs may be a major contributing factor. Since these ILCs are typically induced by barrier cell-expressed IL-25 and IL-33, the cellular sources of these cytokines and their influence on ILC/Th2 cell induction are currently under study.

1433

M2A MACROPHAGES ARE NECESSARY AND SUFFICIENT TO MEDIATE EOSINOPHIL-DEPENDENT IMMUNITY TO FILARIAL HELMINTH INFECTION

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Eosinophils are effector cells in the immune control of tissue dwelling helminths. Whilst eosinophil responses are induced by Th2 adaptive immunity, it is not known how eosinophils are instructed to home from the blood to target migratory stages of parasites. Here we provide evidence from an experimental model of filarial infection (*Brugia malayi* mouse model) that eosinophils are a crucial component of the anti-filarial response that limits establishment of infectious larvae. *B. malayi* infectious larvae also induce M2a macrophage activation (alternative activation)

of tissue resident macrophages, which is further pronounced following vaccination with heat-killed larvae. Absence of a functional interleukin 4 receptor α chain (IL-4Rα) leads to failure of M2a activation, impaired eosinophil recruitment and susceptibility to *B. malayi* establishment to the adult phase. To test the functional relevance of M2a development in the eosinophil larvicidal response, we undertook targeted depletion of macrophages by clodronate liposome (CL) treatment. CL-treatment rendered mice highly susceptible to infection with associated impaired eosinophil recruitment. Add-back of purified M2a into CL-treated WT mice restored both M2a expansion and eosinophil influx. Th2 responses were intact in CL-treated WT mice, suggesting that M2a development directly regulated eosinophil recruitment at the infection site. Consistent with this, an increase in CCL11 transcripts from immune cells derived from the infection site of WT but not IL-4Rα-/- mice was apparent. We therefore tested the direct role of M2a in eosinophil regulation and parasite killing by adoptively transferring purified WT M2a into susceptible severe combined immune-deficient mice (SCID). WT M2a SCID recipients induced a rapid eosinophil response and killing of infectious larvae. In a complementary approach, supplying an exogenous source of IL-4 to condition resident macrophages toward an M2a phenotype at the point of infection rendered SCID mice more resistant to larval establishment. Thus we conclude that M2a conditioning via IL-4Rα is both necessary and sufficient in the absence of additional adaptive immune activation to induce resistance to filarial infection via larvicidal eosinophil recruitment.

1434

ALTERNATIVELY ACTIVATED MACROPHAGES (AAM) IN *SCHISTOSOMA MANSONI* LIVER GRANULOMAS ARE DERIVED FROM MONOCYTES AND ARE PHENOTYPICALLY AND FUNCTIONALLY DISTINCT FROM AAM DERIVED FROM TISSUE MACROPHAGES INDUCED BY *LITOMOSOIDES SIGMODONTIS* INFECTION

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Alternatively activated macrophages (AAM) are induced by helminth infections. We investigated the origins of AAM found in the liver granulomas of mice infected with *Schistosoma mansoni*. CX3CR1GFP/+ mice were used to track monocytes and AAM through a combination of intravital microscopy and flow cytometry. GFP+ monocytes in the liver sinusoids arrest upon encountering parasite eggs in the vessels. GFP+ cells with macrophage-like morphology accumulate around the eggs, are incorporated into hepatic granulomas and express markers of AAM. To determine if Ly6Clow or Ly6Chigh monocytes serve as AAM precursors, we transferred pure populations of these cells from CX3CR1GFP/+ mice into infected congenic mice. Ly6Chigh monocytes extravasated into the tissue more efficiently and upregulate PD-L2 suggesting that they are the source of AAM during *S. mansoni* infection. However, when transferred Ly6Chigh monocytes extravasated into the tissue they became Ly6Clow, suggesting that Ly6Chigh monocytes may transition through a Ly6Clow state when differentiating into AAM. In addition to monocytes, AAM can also be derived from tissue resident macrophages that proliferate during Litomosoides sigmodontis infection. AAM derived from these different sources may be phenotypically or functionally distinct. We find that while both monocyte and tissue derived AAM express high levels of ARG1, YM1/CHI3L3 and FIZZ1/RELMA, tissue derived AAM expressed high levels of F480, but low levels of MR1 and PDL2. In contrast, monocyte-derived AAM were F480int and expressed high levels of MR1 and PDL2. Monocyte-derived AAM upregulate the enzyme RALDH2, have high levels of Aldefluor activity indicating the production of retinoic acid (RA), whereas tissue derived AAM do not. Consistent with RA production, only monocyte derived AAM can promote the differentiation of FoxP3+ CD4+ cells when used to stimulate naïve CD4+ cells. Therefore, monocyte derived and tissue derived AAM are phenotypically and functionally distinct.

1435

MOLECULAR CLONING AND CHARACTERIZATION OF NOVEL GLUTAMATE-GATED CHLORIDE CHANNEL SUBUNITS FROM *SCHISTOSOMA MANSONI*

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Neuronal receptors of schistosomes are attractive targets for drug development because these parasites depend entirely on neuronal modulation to control functions vital to their survival and reproduction. Cys-loop ligand-gated ion channels (LGIC) are proven drug targets in nematodes and arthropods, but are poorly characterized in flatworms. We have previously cloned 3 glutamate-gated chloride channel (GluCl) subunits from *Schistosoma mansoni* (Sm), and characterized them by two-electrode voltage clamp (TEVC) in Xenopus oocytes. Concentration-response relationships revealed that the SmGluCl receptors affinity for glutamate is among the highest reported for GluCl to date, with EC50 values of 6.87- 26.28 μM. In addition, TEVC showed that SmGluCl receptors are insensitive to ivermectin (IVM), indicating that they do not belong to the highly IVM-sensitive GluClα subtype group. These SmGluCl subunits appear to be the only non-acetylcholine Cys-loop LGICs found in *S. mansoni*. Phylogenetic analyses suggested that they belong to a novel clade of flatworm GluCls, which also includes putative genes from other trematodes and cestodes. This flatworm GluCl clade is evolutionarily distinct from the nematode-arthropod and mollusc GluCl clades, and from all GABA receptors. Using confocal microscopy, we showed that SmGluCls are distributed throughout the central and peripheral nervous systems of *S. mansoni*. Further work is in progress to provide a detailed description of SmGluCl distribution in males, females, cercaria and somules. Finally, we have initiated RNAi-based functional studies to assess the roles played by SmGluCls in schistosomes. Altogether, these results provide the first molecular evidence showing the contribution of GluCl receptors to L-glutamate signaling in *S. mansoni*, an unprecedented finding in flatworms. This project has uncovered a completely new aspect of neuronal modulation in flatworms, and brings attention to very appealing new anthelmintic targets which could be used to address the urgent need for new chemotherapeutic options for schistosomiasis.

1436

IGE ANTI-SJ6-8 ANTIBODIES PREDICT RESISTANCE TO REINFECTION WITH *SCHISTOSOMA JAPONICUM*

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Our goal is to discover novel vaccine candidates for schistosomiasis japonica 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 longitudinal treatment-reinfection study conducted in Leyte, The Philippines to identify new *Schistosoma japonicum* antigens associated with resistance. Individuals in our cohort (age 8-30 yrs, n=616) were *S. japonicum* infected at baseline, treated with praziquantel and followed with quarterly stool examination for 12 months. We pooled plasma from 10 resistant (RP) and 10 susceptible (SP) individuals, with careful matching for potential confounders, and performed differential screening experiments using an *S. japonicum* adult worm cDNA expression library. We screened 500,000 clones and identified Sj6-8, a 25 kDa hypothetical protein with an IG domain that is uniquely recognized by antibodies in RP but not SP. We have expressed and purified the immuno-relevant region (aa 20-176) in *E. coli* and designated the protein rSj6-8A. Rabbit anti-Sj6-8A recognized a 75 kDa band in adult worm excretory-secretory products and localized

Sj6-8 to the exofacial surface of the tegument and gastrodermis of adult worms by confocal immunofluorescence analysis and immunogold electron microscopy. We developed a bead-based assay to measure anti-rSj6-8A antibody levels in the entire cohort of volunteers. In repeated measures models, individuals with anti-rSj6-8A IgE levels in the upper quartile (n=140) had 58% lower intensity of reinfection measured 12 months after treatment than individuals with anti-rSj6-8A IgE levels in the lowest quartile (n=140, $P < 0.001$) after adjusting for potential confounders including directly observed water contact, village, age, sex, and baseline intensity of infection. Together, these results validate our field-to-lab-to-field based strategy for the rational identification of vaccine candidates and support Sj6-8 as a novel vaccine candidate for schistosomiasis japonica.

1437

IFN-Γ ELISPOT RESPONSES AGAINST WHOLE SPOROZOITES AND PF ANTIGENS IN VOLUNTEERS IMMUNIZED WITH PROTECTIVE PFSPZ MALARIA VACCINE

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In animals, protective immunity induced by irradiated sporozoites (SPZ) is dependent on CD8+ T cells (mice, monkeys) and IFN-γ (mice). We therefore studied IFN-γ responses in 32 subjects immunized multiple times with escalating doses ($7.5x10^3$, $3.0x10^4$ or $1.35x10^5$ PfSPZ) of radiation-attenuated, purified, cryopreserved *Plasmodium falciparum* SPZ (PfSPZ Vaccine, Sanaria) by IV injection. There was a dose response in regard to protection. None of the volunteers were protected at the lowest total dosage and all were protected at the highest total dosage (R. Seder et al. submitted). This provided the opportunity to begin studying the association between immune responses and protection. Because the antigens involved in protective immunity induced by immunization with the PfSPZ Vaccine are unknown, we used IFN-γ ELISpot assays on freshly isolated PBMC to assess recall responses to pools of overlapping 15-mer peptides representing 5 pre-erythrocytic stage proteins, CSP, AMA1, SSP2/TRAP, LSA1 and CelTOS, comparing these responses to those recalled by PfSPZ or the blood stage antigen PfMSP1 as positive and negative controls, respectively. Responses to each of the 5 Pf antigens were of lower magnitude (25-75 sfc/10^6PBMC) than were responses to PfSPZ. Analysis is ongoing, but in general there was a dose response for PfSPZ and several antigens, most strikingly, AMA1 and SSP2/TRAP. The combination of small numbers and the dose responses make it difficult to assess whether ELISpot responses to any particular antigen were associated with protection, but there was an indication that responses to AMA1 and PfSSP2/TRAP may be so associated. Based on the premise that protective responses targeting multiple antigens could be additive, we summed the responses to the 5 tested antigens, and correlated this with responses to PfSPZ. The two magnitudes were similar, and positively correlated. These preliminary data provide a foundation for prospective studies designed to determine the targets and mechanisms of the high level protective immunity induced by PfSPZ.

1438

IMMUNIZING AGAINST MALARIA BY INDUCING ANTIBODY AND CD8 T CELL MEDIATED PROTECTION

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The malaria vaccine, RTS,S/AS01 is safe and delays the onset of clinical malaria by 30%-50% depending on age group. Protection is thought to be primarily mediated by antibodies against the repeat region and possibly CD4+ T cell responses against the C' terminus of the PfCSP. The vaccine does not induce meaningful CD8+ T cell responses. RTS,S/AS01 is not being considered for preventing malaria in non-immune travelers and elimination campaigns, because its protective efficacy is too low. A vaccine for these indications needs to provide >80% protective immunity for at least 6 months. We hypothesize that by adding highly functional, protective CD8+ T cell responses to antibody responses against the PfCSP, such protective immunity can be achieved. This response should be multifunctional as opposed to the high response obtained with adenovirus based vaccines that have not translated protective efficacy in humans. We are using live-attenuated Listeria monocytogenes (Lm) as a vaccine platform due to its demonstrated properties of effectively stimulating robust, multi-functional, cell-mediated immunity, as a result of its intracellular lifecycle and ability to directly infect, deliver antigen to, and stimulate DCs *in vivo*. We have developed an attenuated Lm-based vaccine platform (Lm ΔactAΔinlB) that has been evaluated in multiple clinical trials in patients with malignant and infectious diseases. This live-attenuated Lm ΔactAΔinlB strain is genetically defined with 2 virulence determinants deleted, resulting in a greater than 1,000-fold attenuation as compared to wild-type Lm, but retaining the immuno-stimulatory potency of the fully virulent wild-type pathogen. We used a prime-boost regimen combining selected molecular adjuvants formulated with recombinant PfCSP protein (rPfCSP) and Lm expressing PfCSP (Lm-PfCSP), to induce PfCSP-specific inhibitory antibodies and CD8+ and CD4+ T cell responses. We will discuss the high levels of inhibitory antibodies as assessed by inhibition of liver stage development and assess long term memory seen with our strategy.

1439

IMMUNE RESPONSES OF RHESUS MONKEYS TO A SELF-ASSEMBLING PROTEIN NANOPARTICLE (SAPN) VACCINE DISPLAYING *PLASMODIUM FALCIPARUM* CSP B- AND T-CELL EPITOPES

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We have previously studied in mice the immune responses induced against *Plasmodium falciparum* circumsporozoite protein (PfCSP) epitopes using a self-assembling protein nanoparticle (SAPN) platform. As a path to testing this vaccine in humans, we conducted safety and immunogenicity studies in rhesus macaques. We hypothesized that an SAPN displaying B- and T-cell PfCSP epitopes would be safe and induce significant responses in macaques with and without the use of an adjuvant. Therefore, we constructed a PfCSP-KMY-SAPN displaying 60 copies of the PfCSP internal repeat sequence $(NANP)_4$ and 60 copies each of three previously identified human MHC-restricted CD8 T-cell epitopes (KPKDELDY, MPNDPNRNV and

YLNKQNSL). Monkeys received four immunizations with the PfCSP-KMY-SAPN with or without the adjuvant GLA-SE. No adverse events developed in any of the animals as a result of immunizations. Antisera and PBMC's were obtained and evaluated by multiple criteria to determine their PfCSP immune specificity. Titer to NANP repeats by ELISA was about $5x10^2$ following immunizations of PfCSP-KMY-SAPN in saline, but increased 20-fold to ~ $1x10^4$ in combination with GLA-SE. Passive transfer of purified IgG from rhesus immunized with PfCSP-KMY-SAPN/GLA-SE prevented infection of 100% of C57Bl/6 mice by a lethal challenge of a transgenic *P. berghei* sporozoite displaying full length *P. falciparum* CSP. Furthermore, serum from these same PfCSP-KMY-SAPN/GLA-SE-immunized monkeys inhibited *P. falciparum* sporozoite infection of primary human hepatocyte cultures by 90%. PBMC were purified and are undergoing evaluation for epitope-specific IFN-γ, IL-2 and TNFα responses. In conclusion, a PfCSP-KMY-SAPN vaccine for malaria was safe and immunogenic in rhesus monkeys. Immune responses to the vaccine were greatly enhanced if the nanoparticle was formulated with the adjuvant GLA-SE.

1440

PROFILING OF ANTIBODIES IN LYMPHOCYTE SUPERNATANTS (ALS) FROM *PLASMODIUM FALCIPARUM* INFECTED PATIENTS IN PERU

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Currently available serologic assays cannot distinguish between circulating antibodies secreted by long-lived plasma cells generated in response to remote infections from antibodies secreted by plasmablasts generated in response to acute or recent infections. To address this technological gap in the context of malaria we developed a high throughput assay to profile Antibodies in Lymphocyte Supernatants (ALS) which are representative of antibodies secreted by circulating plasmablasts. Serum samples and peripheral blood lymphocytes were collected from Peruvian adults with either symptomatic or asymptomatic *Plasmodium falciparum* infection as well as from uninfected controls. Serum samples and supernatants from lymphocyte culture supernatants were probed against protein microarrays containing 500 *P. falciparum* and 500 *P. vivax* proteins. Strong antibody responses were detected in ALS from asymptomatic patients, whereas reactivity in symptomatic patients was much lower, and reactivity in control individuals was negligible. *P. falciparum* antigens differentially recognized by asymptomatic and symptomatic parasitemic individuals were identified. The antibody profiles of the corresponding serum samples were also determined and compared to ALS, and cross-reactivity with the *P. vivax* orthologous was also examined. These data demonstrate the feasibility of separately profiling the antigen specificity of antibodies from plasmablasts resulting from recent exposure, from antibodies circulating in serum that are derived from mature long lived plasma cells. Applied to various study designs involving natural and/or experimental infections with *P. falciparum* and other pathogens, this relatively simple technology will likely provide important insights into the nature of the antibody response to *P. falciparum* and other infections.

1441

DECIPHERING THE EXPRESSED ANTIBODY V GENE REPERTOIRE IN MALARIA

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Individuals living in malaria endemic areas gradually acquire conventional parasite-specific memory B cells (MBC) as well as a large population of atypical MBCs that are associated with chronic infectious diseases, including AIDS. At present, we know little about the molecular mechanisms underlying the generation of conventional and atypical MBCs in response to malaria. To gain insight into these processes we are sequencing the variable (V) gene segments of the immunoglobulin heavy (H) and light (L) chain genes from hundreds of conventional and atypical MBC clones from the peripheral blood of children and adults living in a malaria endemic area of Mali. The analyses of paired V_H and V_L sequences on the clonal level will allow us to determine the germline V_H and V_L gene usage in the conventional and atypical MBC population and the relationship between the two. In addition, the number and the nature of somatic hypermutations in V_H and V_L genes will provide insights to the role of antigen-driven selection in the development of those cell types. By this analysis we aim to gain a better understanding of the generation of both conventional and atypical MBC during the acquisition of antibody-dependent immunity in malaria.

1442

THE PFRH AND EBA INVASION LIGANDS OF *PLASMODIUM FALCIPARUM* ARE IMPORTANT TARGETS OF HUMAN INHIBITORY ANTIBODIES AND FUNCTION TO EVADE NATURALLY ACQUIRED IMMUNITY

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Acquired antibodies are important in human immunity to malaria and can inhibit *Plasmodium falciparum* invasion of erythrocytes, but key target antigens of protective and functional antibodies are largely unknown. Phenotypic variation by *P. falciparum* merozoites can mediate the evasion of inhibitory antibodies, contributing to the capacity of *P. falciparum* to cause repeated and chronic infections. However, antigens involved in mediating immune evasion have not been defined, and studies of the function of human antibodies are limited. We have studied immune responses to *P. falciparum* reticulocyte binding homologues (PfRh1, PfRh2, PfRh4, and PfRh5) and erythrocyte-binding antigens (EBA175, EBA140, and EBA181), which are two families of invasion ligands that play important roles in invasion of erythrocytes and are potential vaccine candidates. We used novel and complementary approaches to determine the importance of PfRh proteins and EBAs as targets of protective human and invasion-inhibitory antibodies, and we defined their role in contributing to immune evasion through variation in function. We evaluated the invasion-inhibitory activity of acquired antibodies from malaria-exposed children and adults using *P. falciparum* lines with targeted disruption of genes encoding different PfRh and EBA ligands in functional assays, and the invasion-inhibitory activity of human affinity-purified antibodies to PfRh and EBA ligands. Furthermore, we examined the association between antibodies to different PfRh and EBA ligands

and protection from malaria in a longitudinal cohort study of children. Considering all data together, our findings provide important evidence that PfRh and EBA ligands are major targets of invasion-inhibitory and protective human antibodies, and that variation in the expression and function of the PfRh and EBAs mediates evasion of acquired antibodies. This knowledge will help to advance malaria vaccine development and understand how the immune response targets multiple invasion ligands to overcome the capacity of *P. falciparum* for immune evasion.

1443

DEMONSTRATION OF ENHANCED STRAIN-SPECIFIC *PLASMODIUM FALCIPARUM* MULTIFUNCTIONAL T CELL CYTOKINE EXPRESSION AMONG MALIAN CHILDREN IMMUNIZED WITH THE FMP2.1/AS02A VACCINE

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Based on *Plasmodium falciparum* (Pf) apical membrane antigen 1 (AMA1) from strain 3D7, the malaria vaccine candidate FMP2.1/AS02A was tested in a Phase 2 clinical trial in 400 Malian children (aged 1-6 years) randomly assigned to receive 3 doses of the AMA1 vaccine or a control rabies vaccine on days 0, 30 and 60. A subset of 10 Pf(-) (i.e., no clinical Pf episodes) and 12 Pf(+) (clinical malaria episodes with parasites with 3D7 or Fab9-type AMA1 cluster 1 loop [c1L]) AMA1 recipients, and 10 controls were randomly chosen for analysis. Peripheral blood mononuclear cells (PBMCs) isolated on days 0, 90 and 150 were stimulated with full-length 3D7 AMA1 and with c1L from strains 3D7 and Fab9 (identical to 3D7 c1L except for amino acid 197) to assess allele-specific cell-mediated responses. T cell expression of INF-γ, TNF-α, IL-2, and/or IL-17A was analyzed by 11-color flow cytometry. Among AMA1 recipients, 19/21 evaluable samples stimulated with AMA1 demonstrated significantly increased levels of IFN-γ, TNF-α and IL-2 derived from CD4+ T cells by D150 compared to 0/10 in the control group (P<0.0001). CD4+ cells expressing both TNF-α and IL-2 were increased in Pf(-) children (median=28.4% of cytokine-expressing cells) compared to Pf(+) children in the AMA1 vaccine group (median=8.6% of cytokine-expressing cells). Low prevalence of double- (IFN-γ+TNF-α+) and triple-positive (IFN-γ+TNF-α+IL-2+) CD4+ cytokine-expressing cells were noted. When PBMCs were stimulated with c1L from 3D7 and Fab9 separately, 5/19 AMA1 recipients with an AMA1-specific CD4+ response had a significant response to one or both c1L. This suggests that AMA1 vaccination induced an AMA1-specific CD4+ response; however, recognition of the vaccine antigen is not dependent upon c1L alone. In summary, AMA1-specific T cell cytokine expression was notably increased in children vaccinated with an AMA1-based vaccine compared to rabies. The possible role of CD4+ TNF-α+IL-2+-expressing T cells in vaccine-induced strain-specific protection against clinical malaria requires further exploration.

1444

DETECTION AND SEMIQUANTITATION OF VENOM AND ANTIVENOM IN THE BLOOD OF TWENTY PATIENTS WITH EVIDENT NEUROTOXIC ENVENOMATION IN GUINEA

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In Guinea, elapids are responsible for about 20% of envenomations. Recent studies have shown that case fatality rate falls between 15 and 30% regardless of treatment. We obtained blood samples from 20 patients who presented typical neurotoxic syndromes. All patients were treated with 40 ml of antivenom neutralizing the main species of Elapidae in the region: *Dendroaspis polylepis* (Dp), *D. viridis* (Dv), *Naja melanoleuca* (Nm) and *N. nigricollis* (Nn). Blood samples were spotted onto Guthrie paper before antivenom treatment (hour 0, H0) and two hours after antivenom administration (H2). The samples were analyzed by a custom sandwich ELISA for venom of each of the four species using rabbit antibodies purified against the venom of each species by affinity chromatography and adsorbed against the other three species. The presence of antivenom was also established for all samples. Five patients died. Samples on H0 were missing for 2 patients and samples on H2 were missing for another 2. Of the 18 H0 samples tested, a clear and high venom signal was detected in 4: 2 patients who died were strongly positive for Dp, including a patient who died before treatment, 1 patient was strongly positive for Dv (who also died) and one patient who survived was strongly positive for Nn. Antivenom was detected in the H2 samples of 14 patients. For the 2 patients for whom a clear venom signal was detected at H0 and for whom H2 samples were available, residual venom was detected using an ELISA assay based on immunopurified horse antibodies. A competition ELISA test in which venom was titrated with increasing amounts of antivenom showed that the venom detected by means of this ELISA assay is likely to be free residual venom. The ELISA assays performed on samples spotted and dried on Guthrie paper are robust, very specific but not very sensitive. They have nonetheless permitted, for the first time, an immunodiagnosis of the species of African elapid causing envenomation in 4 of 18 patients, including those 3 (of 5) who died during the study. They have also shown that residual "free" venom was present in two patients after antivenom administration, suggesting that the dose of antivenom administered may have been insufficient to completely neutralize the venom present in some of these victims of snakebite.

1445

USE OF JAPANESE ENCEPHALITIS VACCINE IN U.S. TRAVEL MEDICINE PRACTICES IN GLOBAL TRAVEPINET

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Japanese encephalitis (JE) vaccine is recommended for high-risk travelers to Asia and the western Pacific. Few data regarding the use of this vaccine in clinical practice are available. We evaluated international travelers to JE-endemic regions who were seen at U.S. Global TravEpiNet (GTEN) sites between September 2009 and August 2012, when IXIARO was in use. We categorized travelers as higher or lower risk for JE based on the destination country, travel plans, and duration of travel. We compared the demographic and clinical features of higher and lower JE risk travelers and

performed multivariable analyses to identify factors that were associated with travelers not being offered or declining the JE vaccine. We identified 711 higher JE risk travelers and 7,578 lower JE risk travelers in our analysis. Higher JE risk travelers were younger than lower JE risk travelers (median age 29 years vs. 40 years, p<0.001) and traveled for longer durations of time (median 50 days vs. 14 days, p<0.001). 43% of higher JE risk travelers were offered the JE vaccine, and 62% of these travelers accepted it. Short time to departure, rural travel, travel to visit friends and relatives, leisure travel, and travel for humanitarian service work were each independent predictors of declining the JE vaccine. Additionally, 40% of higher JE risk travelers were judged by their clinician to not require the JE vaccine. Travel to visit friends and relatives, leisure travel, travel to India, and travel to China were independent predictors of a higher JE risk traveler not being offered the JE vaccine. Clinicians did not recommend the JE vaccine to many travelers who met the indications offered by the Advisory Committee on Immunization Practices, and there are disparities with regard to subpopulations that receive the vaccine.

1446

EOSINOPHILIA AS A POTENTIAL SURROGATE FOR THE DIAGNOSIS OF STRONGYLOIDIASIS IN AN IMMIGRANT POPULATION AND THE UTILITY OF ABSENT SS-NIE ANTIBODIES AS A BIOMARKER FOR CURE

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Determining the cause of persistent eosinophilia in immigrants to the United States can be hampered by costs needed to evaluate suspected parasitic infections. Thus, diagnosing eosinophilia-causing helminth infections by stool examination or serology is often beyond the means of community health clinics that commonly serve immigrant populations. To define the causes of persistent eosinophilia among an immigrant population seen at a single community free health clinic, 54 patients (originally from Central and South America, Africa, Asia and the Middle East) who arrived in the United States 1-27 years (median 7 years) previously--were found to have an absolute eosinophil count (AEC) >500/uL and were referred to the National Institutes of Health for further testing. Of the 54 referred patients, 43 (80%) had positive *Strongyloides stercoralis* (Ss)-specific serology. 1/43 (2%) also had schistosomiasis, 3/43 (7%) hookworm, and 2/43(5%) trichuriasis. There were no differences in baseline eosinophil counts and serum IgE levels between those with Ss and the 11/54 without (probably reflecting the referral criteria). All patients with a definitive parasitologic diagnosis received ivermectin and (when appropriate praziquantel and/or albendazole) treatment and followed over the course of a year. Not unexpectedly, there was a dramatic and significant (p<0.0002) decrease in AEC following treatment with all returning to normal levels by 1 year. IgE levels also fell dramatically following treatment. Most importantly, antibodies to the *Strongyloides*-specific recombinant antigen (Ss-NIE) using a luciferase immunoprecipitation assay (LIPS) also became negative in all those with Ss treated successfully with ivermectin. Thus, in community clinics that provide health care to immigrants well after arrival in the United States, an AEC can be used as a surrogate for stool examination, and serology may be a trigger for empiric treatment when testing is limited by cost. If available, newer serologic tests may replace insensitive stool examinations as tests of cure.

1447

RISK FACTORS AND SEROPREVALENCE OF *TRYPANOSOMA CRUZI* INFECTION IN TEXAS

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Chagas' disease has emerged as an important neglected tropical disease in the United States; particularly in Texas. Chagas' disease is caused when the parasite *Trypanosoma cruzi* (*T. cruzi*) is transmitted to humans by a *Triatominae* insect. One-third will develop chronic infection that can result in cardiac myopathy and death. This study aimed to determine risk factors and estimate disease burden of Chagas disease in Texas. Data was collected from five major blood centers in Texas on those tested for *T. cruzi* from 2008-2012. We only included original donations tested from each donor, and duplicate donations were excluded for seroprevalence analysis by stratification. Risk factors were analyzed by zip-codes with and without a reported case to the Chagas' Biovigilance Network and/or a major Texas blood center. We found 1 per 3,500 population were positive for *T. cruzi* in Texas. Seroprevalence was similar between genders. Infection rate increased with age with ages 41-50 (40 per 100,000) and 51+ (41 per 100,000) having the highest infection rate. As expected, Hispanics had the highest infection rate (43 per 100,000). Caucasians (14 per 100,000) and African Americans (24 per 100,000) had the lowest infection rates. We calculated a total cost to society of $215 million for these cases. *T cruzi* positive cases were significantly more likely to live in zip-codes that have a higher percentage of foreign born residents (p<0.001) and urban land use (p<0.001). In conclusion, blood Centers are an important component in understanding *T. cruzi* transmission in Texas. Approximately 1 per 3,500 blood donors test positive for *T. cruzi*. Chronic cases accrue $215 million in lifetime societal cost to Texans. Minorities, urban areas, and areas with high foreign born population are at highest risk for *T. cruzi* infection.

1448

SPATIAL DISTRIBUTION OF PODOCONIOSIS IN RELATION TO ENVIRONMENTAL FACTORS IN ETHIOPIA: A HISTORICAL REVIEW

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An up-to-date and reliable map of podoconiosis is needed to design geographically targeted and cost-effective intervention in Ethiopia. Identifying the ecological correlates of the distribution of podoconiosis is the first step for risk and distribution maps. The objective of this study was to investigate the spatial distribution and ecological correlates of podoconiosis using historical and contemporary survey data. Data on the observed prevalence of podoconiosis were abstracted from published and unpublished literature into a standardized database, according to strict inclusion and exclusion criteria. In total, 10 studies conducted between 1969 and 2012 were included through structured searches, and data were available for 401,674 individuals older than 15 years of age from 229 locations. A range of high resolution environmental factors were

investigated to determine their association with podoconiosis prevalence, using logistic regression. The prevalence of podoconiosis in Ethiopia was estimated at 3.4% (95% CI: 3.3%-3.4%) with significant regional variation. We identified significant associations between altitude, mean annual Land Surface Temperature (LST), mean annual precipitation, topography of the land and fine soil texture and high prevalence of podoconiosis ($p<0.001$). The derived maps indicate both widespread occurrence of podoconiosis and a marked variability in prevalence of podoconiosis, with prevalence typically highest at altitudes >1500 m above sea level (masl), with >1500 mm annual rainfall and mean annual LST of 19-21°C. No (or very little) podoconiosis occurred at altitudes <1225 masl, with annual rainfall <900 mm, and mean annual LST of >24°C. Podoconiosis remains a public health problem in Ethiopia over considerable areas of the country, but exhibits marked geographical variation associated in part with key environmental factors. This is work in progress and the results presented here will be refined in future work.

1449

DISCOVERING THE PATHOGENS OF CENTRAL NERVOUS SYSTEM INFECTION IN NEPAL

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Central nervous system (CNS) infection is one of the common causes of hospital admission in Nepal. Due to the absence of specific tests to diagnose the definitive cause of meningitis, the treatment is often empirical. The condition is more challenging when there is prior use of antibiotics. Such-conditions alter the possible outcomes, which ultimately affects treatment and management. Therefore, the aim of this study is to find the possible etiological agents responsible for meningitis in adults in Nepal. We conducted a prospective hospital based study to identify the possible pathogens of CNS infections in adults admitted in Patan Hospital from February 2009-April 2011. The pathogens of CNS infections were confirmed in cerebrospinal fluid (CSF) using molecular diagnostics, culture (bacteria) and serology. 87 patients were recruited for the study and the etiological diagnosis was established in 38% (n=33). The bacterial pathogens identified were Neisseria meningitidis (n=6); Streptococcus pneumoniae (n=5) and *Staphylococcus aureus* (n=2) in 13/87(14%). Enteroviruses were found in 12/87 (13%); Herpes Simplex virus (HSV) in 2/87(2%). IgM against Japanese encephalitis virus (JEV) was detected in CSF of 11/73 (15%) tested samples. In conclusion, our study is the first (RT) PCR and serology based CSF analysis from Kathmandu, Nepal that attempts to indentify the causative organisms of infectious syndromes of the central nervous system in adults. JEV and enteroviruses were the most commonly detected pathogens.

1450

SEVERE MALARIAL ANEMIA IS ASSOCIATED WITH LONG-TERM NEUROCOGNITIVE IMPAIRMENT

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Cerebral malaria (CM) is associated with long-term cognitive impairment in children 5 years of age and older. No prospective studies have assessed cognitive impairment in children with CM <5 years of age, or in children with severe malarial anemia (SMA), a more common manifestation of severe malaria that is estimated to affect > 5 million children annually. Children <5 years of age who presented to Mulago Hospital, Kampala, Uganda, with CM (n=80) or SMA (n=86) were assessed for overall cognitive function, attention, and declarative memory one week after discharge and 6 and 12 months later. Age-adjusted z-scores for each domain were generated from the scores of 61 healthy community children (CC), who were also tested at enrollment and 6 and 12 months later. Groups were compared using mixed linear models. For the full one-year period of follow-up, children with CM had significantly worse scores than CC in overall cognitive function (-1.00 vs -0.12; $P<0.0001$), attention (-0.51 vs -0.06; $P=0.004$), and declarative memory (-0.41 vs 0.04; $P=0.0003$). Children with SMA also had significantly worse scores than CC in overall cognitive function (-0.52 vs -0.12; $P<0.0001$) and attention (-0.25 vs -0.06; $P=0.03$), but not declarative memory (-0.03 vs 0.04; $P=0.99$). Scores for overall cognitive function and attention did not differ significantly between children with CM vs. SMA. In children <5 years of age, CM is associated with long-term impairment in overall cognitive function, attention, and declarative memory, and SMA is associated with long-term impairment in overall cognitive function and attention. SMA may be a major cause of long-term neurocognitive impairment in children in sub-Saharan Africa.

1451

EPIDEMIOLOGICAL, CLINICAL AND LABORATORY DESCRIPTION OF ONCHOCERCIASIS IN AN AREA OF HIGH PREVALENCE - JIMMA, ETHIOPIA, 2013

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Onchocerciasis, transmitted by blackflies, still infects at least 37 million people worldwide, but elimination of onchocerciasis through mass drug administration (MDA) with ivermectin is feasible in parts of Africa. However, evidence-based tools to evaluate program endpoints are lacking. Clinical specimens characterized with epidemiologic, clinical, and laboratory data were collected and analyzed to evaluate the existing diagnostic tests for onchocerciasis and to identify the best tests to measure programmatic endpoints. Five-hundred specimens were collected in three onchocerciasis-endemic areas in Jimma, Ethiopia, where one round of ivermectin MDA had been given five months before the study. Laboratory analysis in country included blood smears to detect *Loa loa* and *Mansonella perstans*, immunochromatographic card tests (ICT) for filariasis, and skin snip examination for *Onchocerca volvulus*. Plasma, serum, blood smears, dried blood spots, and preserved skin snips were sent to CDC for further analysis. The median age of participants was 45 (range 6-90 years); 276 (55%) were male. Though only 57 (11%) participants reported living near a river, 244 (49%) spent the majority of the day near rivers where blackflies typically bite. Eight (2%) participants had *O. volvulus* microfilaria present in the anterior chamber (N=2) or cornea (N=6). At least one skin nodule was noted in 319 (64%) participants (range 1-11); 312 (62%) had other onchocercal skin manifestations; 74 (15%) had evidence of lymphedema. The skin snip was positive for *O. volvulus* in19 (4%) participants, with a mean load (average of both snips) among those with at least one positive snip of 19.5 microfilaria/slide (range 0.5-180); 15 (3%) had positive ICTs. The paucity of positive skin snips despite the high prevalence of nodules is unexplained and needs further investigation. *Wuchereria bancrofti* may be co-endemic in the areas studied. Additional laboratory evaluation is pending. The performance of these tests in the African context will help determine their use in the evaluation of elimination program endpoints.

1452

COMPLEMENTARY USE OF COMPREHENSIVE SURVEILLANCE AND TRANSMISSION ASSESSMENT SURVEYS FOR ASSESSING PERSISTENCE OF LYMPHATIC FILARIASIS IN SRI LANKA FOLLOWING MASS DRUG ADMINISTRATION

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The Sri Lankan Anti-Filariasis Campaign (AFC) provided mass drug administration (annual diethylcarbamazine plus albendazole) according to WHO guidelines to some 10 million people in 8 endemic districts between 2002 and 2006. All districts met WHO criteria for lymphatic filariasis (LF) elimination in 2008, but spot surveys showed low-level persistence of microfilaremia (Mf) in some sentinel sites. Comprehensive surveillance of suspected hotspots (in 2 public health inspector areas per district) was initiated in early 2010, and WHO recommended TAS surveys were conducted in 2012-13. Comprehensive surveillance included community surveys for Mf and filarial antigenemia (ICT), school surveys for ICT and anti-filarial antibodies (Bm14 ELISA) in children 6-8 years of age, and mosquito surveys to detect filarial DNA in *Culex* mosquitoes collected by gravid traps (molecular xenomonitoring, MX). TAS surveys involved ICT testing of ~1,500 children in 30 to 35 randomly selected schools in each evaluation unit. Provisional targets for LF elimination in hotspot surveys were <0.5% for Mf (community surveys), <2% for ICT (community), <2% for antibody in first grade primary school children, and <0.25% for filarial DNA in mosquitoes. We now report results from 13 hot spot surveys that were conducted in 6 formerly endemic districts. Community Mf and ICT prevalence rates were between 0-0.9% and 0-3.4%, respectively. ICT rates in school children were < 1% in all 13 sites, but antibody rates in school children exceeded 2% in 9 sites. Filarial DNA rates in mosquitoes exceeded the target rate in 7 of 13 study sites, and all of these LF indicators exceeded our targets in one site. Thus, many hot spots had evidence of low level persistence of LF some 6 years after MDA. TAS survey results showed that ICT rates in primary school children satisfied WHO targets in all 8 districts. These results suggest that antibody testing of children and MX are more sensitive tools for detecting low-level persistence of filariasis in communities than TAS. We recommend using enhanced surveillance tools to complement TAS surveys for post-MDA surveillance. We also recommend close follow-up for areas that failed to meet elimination targets to determine whether further intervention is required in these areas.

1453

ACTIVE TRANSMISSION OF FILARIASIS IN ZANZIBAR AFTER MDA HAD BEEN STOPPED FOR FIVE YEARS

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The Global Programme to Eliminate Lymphatic Filariasis (GPELF) recommends annual mass drug administration for 4-6 years to interrupt transmission of the disease. Zanzibar, in the United Republic of Tanzania, was the first country to complete five rounds of treatment using a combination of albendazole and ivermectin at 100% geographic coverage and achieving effective coverage rate of over 65% during all five years. MDA implemented through filarial prevention assistants (FPAs) selected that were resident in the communities and aware of public health activities to various degrees. Total treatment coverage averaged from 70 to 80% in all five rounds mainly due to a very effective social mobilization programme. Impact assessment at two sentinel sites showed that the prevalence and intensity of microfilaria decreased significantly after the first round of MDA and the decline continued after subsequent MDA rounds. MDA was stopped in 2006 after sentinel site surveys revealed prevalence below 1%.In early 2012, transmission assessment surveys (TAS) were conducted to determine if transmission of LF had been interrupted on the two islands. The TAS surveys involved a total of 72 schools; 36 from each of the two Evaluation Units (EUs) in Pemba and Unguja islands. A total of 1298 children were surveyed on Pemba where 70 (5.4%) were found to be positive. In Unguja, 19 (0.95%) of the 1980 pupils tested were positive. The EU on Pemba exceeded the critical cut-off of 18, thereby failing the TAS criteria and implying that transmission had resumed. On the other hand the number of positives from Unguja just fell short of 20, suggesting that the level of exposure, though high, may not be sufficient to sustain transmission. Based on the TAS results it was recommended that MDA be restarted on both islands in 2013 because of the efficiency of the *Culex* vector. Another TAS will be conducted after two rounds. This study confirms the recommendation that effective surveillance and possible continued actions after the achievement of the elimination target may be required to prevent re-establishment of transmission

1454

TRANSMISSION OF ONCHOCERCIASIS IN CENTRAL NIGERIA: ONGOING TRANSMISSION OR DISEASE ELIMINATION?

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Mass drug administration (MDA) with ivermectin is the WHO recommended strategy for control of onchocerciasis. Recent evidence has shown that after 15-17 years of treatment, elimination of the disease in Africa may be possible. In Plateau and Nasarawa states in North-Central Nigeria, MDA has been ongoing since 1991. Since 2000, albendazole has been co-administered with ivermectin for treatment of lymphatic filariasis (LF), which is co-endemic. In 2009, 5 districts were determined to have stopped LF transmission. We set out to evaluate the status of onchocerciasis transmission in these 5 districts to determine onchocerciasis transmission had also been interrupted. Using the 2001, WHO criteria for elimination of onchocerciasis, we sought to achieve a microfilariae (MF) and seroprevalence of <1/1,000 infected individuals and a rate of infective blackflies of <0.05/1,000. We evaluated adults and children in six sentinel sites and children only in eight spot-check villages. Skin snips, blood spots, and nodules were collected and fly catches were conducted at six river sites. We sampled a total of 5,182 persons: 4,441 children ages 3 to 12 and 746 adults ≥20 years in 14 communities. In adults, Mf prevalence had decreased 99.3% from a mean baseline of 43.0% to 0.27% (p<0.001). In children, no Mf were detected but a seroprevalence of 0.16% (n=7, 0.32% upper 95%CI) was found. A total of 1,568 blackflies were assessed in six capture sites. While no infective larvae were found, the number of flies caught was insufficient for determining whether transmission has been interrupted. In conclusion, current criteria from APOC use parasitologic and entomologic indicators for determining elimination status. In areas where the blackfly vector is less abundant, however, the number of flies needed to definitively decide may not be possible to obtain in a timely manner. In Plateau and Nasarawa states, we have found that, while we meet the parasitologic criteria, we cannot achieve the necessary number of flies needed to definitively determine if transmission has been interrupted despite the fact that no infective flies have been found. In this case, we have opted to include the 2001 WHO criteria to examine serologic evidence in children and have found that transmission may be ongoing.

1455

MALARIA AND FILARIASIS COINFECTIONS IN PAPUA NEW GUINEA

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Malaria and lymphatic filariasis (LF) elimination programs are predicated on efficient and effective surveillance and monitoring tools. Simultaneous detection of all four primary malaria species and *Wuchereria bancrofti* (Wb) using a post-PCR oligonucleotide ligation detection reaction-florescent microsphere assay (LDR-FMA) has been recently demonstrated in samples from the Dreikikir region of Papua New Guinea where malaria and filariasis are co-endemic and transmitted by Anopheline mosquitoes. In this setting mass drug administration has been deployed against LF beginning in the mid 1990s and long-lasting insecticide-treated nets were distributed in 2009. The present study evaluates a population of 2700 individuals in this region to quantify co-infection dynamics and characterize the complex epidemiology of these important parasitic diseases. Overall, our results showed that 84.1% of the individuals tested were assay-positive for at least one malaria species and 13.4% were positive for Wb. Among individuals infected with *Plasmodium* species parasites, 38.0%, 29.4%, 13.4%, and 3.3% of individuals were infected with 1, 2, 3, or 4 species of malaria, respectively. Diagnosis of Wb infection prevalence did not differ significantly according to the quantity of malaria species co-infections (p=0.350), even after stratifying for intensity of Wb infection (using ICT card grade or microfilaremia) or malaria LDR-FMA optical densities. Furthermore, Wb prevalence was not significantly different among malaria positive or negative individuals (13.6% vs 12.0%, p = 0.380). Interestingly, we observed that Wb infections were slightly more common in malaria infected (37.6) vs. uninfected individuals (28.0%) (p=0.044) among the subset of individuals residing in a geographic area traditionally characterized as having higher LF transmission prevalence. 35.6% of individuals in the higher LF transmission site and 7.6% of individuals in the lower LF transmission site were Wb positive by LDR-FMA whereas malaria prevalence was similar across sites (79.5% and 85.6%, respectively). Simultaneous multiple parasite detection such as LDR-FMA may be useful to integrated disease monitoring and elimination strategies.

1456

EVALUATION OF TEN YEARS IMPACT OF IVERMECTIN TREATMENT FOR ONCHOCERCIASIS ON LYMPHATIC FILARIAIS: A CASE STUDY FOR THREE OVERLAPPING DISTRICTS IN TANGA REGION, TANZANIA

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Tanzania is endemic with 5 of the PCT targeted NTDs namely Lymphatic Filariasis - LF, Onchocerciasis, Trachoma, Schistosomiasis and Soil Transmitted Helminthiasis - STH. MDA activities implemented in phases in various implementation units. Tanga region has been implementing consecutive Ivermectin MDA for Onchocerciasis control since 2000 and Ivermectin + Albendazole MDA for LF since 2004 and has completed over 5 effective Mass Drug Distribution rounds and the average coverage being above 65%. The study aim was to evaluate the impact of 10 rounds of Ivermectin treatment for Onchocerciasis on Lymphatic Filariais. The Survey districts were Lushoto, Muheza and Korogwe. Six sites for lymphatic filariasis were selected from the participating districts. One village with high prevalence of Onchocerciasis and another one with high prevalence of Lymphatic Filariasis were selected from each district. All hamlets in selected villages were surveyed. A cluster survey was applied involving communities. For LF survey, eligible population were individuals aged 5 years and above. A systematic random sampling of households was done to get 600 participants from each village. Enrolled individuals from the community were tested for Circulating Filarial Antigen (CFA) using ICTs. 100microlitres of blood sample from participants was collected from a finger prick, ICT was done and results provided after 10 minutes. For all ICT positive results night blood was collected between 10pm and midnight and microfilaria(mf) count was done using counting chamber technique. A total of 1887 people participated in the LF survey, 1020 (54.1%) were females and 867(45.9%) were males. All of the participants were tested for CFA with ICT, 40(2.1%) were ICT positive. Mf count was done to 34 of the ICT positives and 6 were positive. Results indicate that LF is still prevalent in the evaluated districts with different CFA prevalence levels and thus MDA should continue for few more round before conducting Transmission assessments survey (TAS).

1457

FORECASTING DEMAND FOR ONCHOCERCIASIS TREATMENT TO ACHIEVE ELIMINATION AND ERADICATION

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Recent evidence indicates that mass onchocerciasis treatment with ivermectin can interrupt transmission and eliminate the parasite in endemic foci if high treatment coverage greater than 65% is maintained for a decade or more. Realistic estimates of the coverage levels needed to achieve elimination and eradication can inform donors' investment decisions. We estimated the number of treatments needed to go from the current control level to elimination and eradication of onchocerciasis as the geographic and therapeutic coverage is scaled up. Scenarios were developed assuming treatment continues until the population of female adult worms is reduced to a threshold where it is expected to irreversibly move to its demise. The number of treatments required, from 2012 to 2040, was predicted using historical data. The years of introduction and the coverage rates were collected from APOC treatment database, while for untreated areas they were predicted based on the expected launching year of APOC's budget plan, political and operational challenges, nodule prevalence, at-risk population, and average treatment coverage rates in the country. The treatment duration was predicted based on the existing results of a micro simulation model (ONCHOSIM). The estimated number of treatments needed in sub-Saharan countries is around 75 million in 2012. If the current strategy continues, the annual demand will increase to around 120 million by 2030. In the elimination scenario, the annual demand would decrease to around 30 million by 2030 because many endemic areas will not require treatment any longer. The treatment demand is expected to further decrease to less than 12 million by 2030 in the eradication scenario, whereby challenging areas with post-conflict situation or co-endemicity with loiasis will be treated with locally tailored approaches, so that treatment won't be required in the end. The results show that scaling up ivermectin coverage to achieve elimination and eradication would eventually lead to potential cost savings.

1458

WHAT HAVE WE LEARNED FROM GWAS STUDIES OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE*?

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Driven by advances in marker availability and screening technologies, the last six years has seen an explosion of genome-wide association studies (GWAS) in humans, most aimed at detecting genetic variants linked to common diseases. With a well-assembled genome, unquestioned medical

importance, and dwindling insecticide susceptibility a major threat to control, *Anopheles gambiae* insecticide resistance represented a natural target for the earliest GWAS in insects. Employing sequentially increasing numbers of markers, our studies have confirmed known causal variants and provided new field-applicable markers for pyrethroid resistance, but, in common with GWAS in humans, have yet to discover the novel variants of major effect that were initially anticipated. We highlight a number of design-related issues, recognition of which will aid future work. These include: (1) poor performance of designs inherited from human studies for mosquitoes and especially for insecticide resistance; (2) closer attention to phenotype definition, and (3) greater attention to environmental variation as a source of 'missing' trait heritability. With appropriate design alterations, GWAS can benefit considerably from imminent advances such as quality genome assemblies for multiple *Anopheles* disease vectors and a validated SNP-call database for *An. gambiae*.

1459

A SINGLE MUTATION IN THE GLUTATHIONE-S TRANSFERASE GENE (GSTE2) IS RESPONSIBLE FOR INSECTICIDE RESISTANCE IN THE MAJOR MALARIA *ANOPHELES FUNESTUS*

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Metabolic resistance to insecticides is the biggest threat to the continued effectiveness of existing malaria vector control interventions. But its underlying molecular and genetic basis, crucial for successful resistance management, remains poorly characterised. In this study, using a genome-wide transcriptional analysis, we showed that the up-regulation of the glutathione-S transferase gene GSTe2 is strongly associated with DDT resistance. Using a GAL4/UAS transgenic expression of this gene in *Drosophila*, we demonstrated that over-transcription of this gene alone was necessary and sufficient to confer DDT resistance but more importantly also cross-resistance to pyrethroids. We showed that besides quantitative differences, qualitative changes in GSTe2 were also significantly contributing to the high DDT resistance as *In vitro* metabolic assays demonstrated that the resistant allele was more active in metabolizing DDT than the susceptible alleles. For the first time in mosquitoes, we identified an amino acid change (L119F) that strongly associates with DDT resistance and designed a molecular diagnostic assay that accurately detects the resistance in field populations. Structural analysis of the GSTe2 indicated that L119F located in the DDT-binding pocket confers the high DDT resistance by significantly increasing the size of the DDT binding cavity allowing more binding of the DDT molecule leading to its increased metabolism. The distribution of this L119F mutation across Africa shows a strong correlation with known patterns of DDT resistance. Furthermore, we showed that GSTe2 is under strong directional selection in resistant populations, and a restriction of gene flow is observed between African regions, enabling the prediction of the future spread of this resistance. This study represents a comprehensive and detailed dissection of the genetic, molecular and structural basis of metabolic resistance to insecticides and provides the first resistance marker for metabolic resistance in mosquitoes.

1460

INSECTICIDE RESISTANCE IN SYMPATRIC *ANOPHELES GAMBIAE* AND *AN. ARABIENSIS* FROM UGANDA: EVIDENCE FOR EVOLUTIONARY CONVERGENCE IN THE RESISTANCE ASSOCIATED GLUTATHIONE-S TRANSFERASE GSTE4

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In Tororo, eastern Uganda, a high malaria transmission setting, and Jinja (approximately 110km from Tororo) where transmission intensity is lower, we have demonstrated extensive resistance to pyrethroid insecticides. This in the absence of universal distribution of ITNs or any IRS programme. In whole-genome microarray analysis of pyrethroid resistant *Anopheles gambiae* (Tororo) and *An. arabiensis* (Jinja) the glutathione-S transferase GSTe4 is significantly up-regulated in both species, suggestive of a role in the resistance phenotype. Where *An. gambiae* and *An. arabiensis* are sympatric we find a low level (0.22% *N*=7,202) of hybrid samples, and using a multiplex SNP array we demonstrate that in addition to F1s there are individuals which are the progeny of advanced backcrossing. This raised the possibility that introgression of selected genes, such as an insecticide resistance associated GSTe4 variant, may have occurred. Sequencing of GSTe4 haplotypes was not supportive of our hypothesis of introgression of GSTe4 variants but comparison of non-synonymous and synonymous changes were suggestive of marked functional constraints/ sequence convergence of GSTe4. Biochemical assays, showed that whilst GSTe4 does not actively metabolise pyrethroids, it is strongly inhibited by them, indicative that GSTe4 may play a role in sequestering insecticides in both species. This region of Uganda is experiencing marked flux in resistance status and species composition. We are using next generation whole genome sequencing of *An. gambiae* and *An. arabiensis* in order to better understand the consequences of hybridisation for the transfer of traits relevant to insecticide resistance between these important vectors.

1461

A UNIQUE MUTATION ON THE *ACE*-1 GENE OF THE MALARIA VECTOR *ANOPHELES ALBIMANUS* PROVIDES EVIDENCE FOR BALANCING SELECTION IN AN AREA OF HIGH INSECTICIDE RESISTANCE IN PERU

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Acetylcholinesterase (AChE) insensitivity has previously been associated with resistance to organophosphate (OP) insecticides in arthropods. A single point mutation on the *ace-1* gene (G119S) has been identified in three anopheline species, including the New World malaria vector *Anopheles albimanus*. High levels of resistance to multiple classes of insecticides have recently been detected in the local *An. albimanus* vector population along the NW coast of Peru. To identify the mechanisms of resistance, the abdomens of 77 engorged females were excised and DNA was extracted, while the heads and thoraces of these individuals were used for biochemical analyses. Elevated levels of AChE insensitivity were detected in the biochemical assays, suggesting that this was a likely mechanism of resistance. A species-specific primer set was designed to amplify the region of the *ace-1* gene that includes the G119S mutation site. Sequencing the region showed that the individuals were highly polymorphic, with all individuals being heterozygous (G/T) at the first base. An additional, novel polymorphism was identified at the adjacent locus, where the individuals were all either heterozygous (G/C; n=63) or homozygous (C/C; n=14). The potential amino acids for individuals heterozygous at both *loci* are glycine (susceptible), serine (resistant), cysteine and alanine. For homozygous individuals at the second base, the only potential amino acids are serine and alanine, suggesting this

novel substitution may be associated with greater AChE insensitivity. This hypothesis is supported by analysis of biochemical and genetic data from the same individuals, which indeed suggests that individuals homozygous at base two presented higher levels of AChE insensitivity than heterozygotes. The G119S mutation appears to have arisen independently in this population, as the polymorphisms that result in serine are unique to what has been previously described. The occurrence of heterozygotes at 2 *loci* suggests that balancing selection could be the driving force behind the maintenance of OP resistance in this population.

1462

INSECTICIDE RESISTANCE SELECTION DRIVES GENETIC DIFFERENTIATION AMONG *AEDES AEGYPTI* FROM YUCATAN

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The mosquito *Aedes aegypti* is the main vector of dengue viruses. Population reduction involves removal of larval breeding sites and uses insecticides for larval and adult control. In Mexico, permethrin has been used for mosquito control over the last 12 years and widespread resistance has been reported. Knockdown and mortality rates obtained by cone assay were highly variable among Yucatan collections and are highly correlated with point mutations in the voltage gated sodium channel gene (VGSC). We sought to determine if permethrin pressure reduces gene flow by comparing SNP variation at neutral gene markers and with variation at markers putatively associated with insecticide resistance. We tested for patterns of gene flow among 27 collections from Yucatan made in 2011. Three groups of nested collections were made around Merida and five collections were in towns outside Merida. A total of 1,301 mosquitoes were genotyped using 13 single nucleotide polymorphism markers (SNPs). Eight SNP's were in putatively insecticide neutral genes: amylase, apyrase, gluco-phosphate isomerase, early trypsin, vitellogenic carboxypeptidase precursor, chymotrypsin and maltase. Two SNPs were in the VGSC gene (C1534 and I1016), two were in cytochrome P_{450} genes (CYP9J32 and CYP9J29) and one was in a carboxyl/choline esterase gene (CCEae1C). F_{ST} for neutral SNP's was low (0.012 - 0.063) and F_{ST} for potential insecticide metabolic genes were similar (0.031- 0.049). However, F_{ST} for SNP's at the VGSC were much higher (0.222 and 0.135 for C1534 and I1016, respectively). AMOVA among all *loci* indicated little variation (3%) among collections from different cities. However, locus by locus analysis showed that C1534 and I1016 cause 22 and 13% of the variation among cities, respectively. In the face of high effective migration rates, local insecticide selection pressure created large variation in VGSC mutations.

1463

THE RAPID SELECTION OF PYRETHROID RESISTANCE IN *ANOPHELES GAMBIAE* IN A SINGLE YEAR: AN INVESTIGATION INTO THE UNDERLYING CAUSES AND POTENTIAL IMPACT

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Resistance to pyrethroids was first reported in *Anopheles gambiae* from the rice growing region of Vallee de Kou in Burkina Faso over 14 years ago. The proportion of resistant mosquitoes has steadily increased since then and it is now rare to find any mosquitoes from this region surviving standard WHO susceptibility diagnostic dose assays. However, without data on the magnitude of resistance it is difficult to predict the impact that this may have on malaria vector control. To address this, we determined the LT50 of the predominant vector from Vallee de Kou, An *gambiae* M form, in 2011 and again in 2012. Remarkably, in just one year, the LT50 had increased > 10 fold. This dramatic increase in the strength of resistance was not accompanied by an increase in kdr frequency, as the frequency of the 1014F allele was >0.8 in both years and the 1575Y allele decreased slightly between the years to 0.27 in 2012. However, using a stringent microarray experiment, with comparisons with multiple susceptible strains, we identified a number of detoxification genes strongly correlated with resistance to deltamethrin. Expression of a subset of these genes, including cytochrome P450 and cuticular genes increased significantly between 2011 and 2012 and may explain the dramatic increase in resistance observed recently. Data on the impact of this very strong resistance phenotype on the efficacy of long lasting insecticide treated bed nets in use in the region, obtained using both cone bioassays and experimental huts will also be presented.

1464

DISSEMINATION OF A POTENT PUPACIDE BY ADULT *AEDES AEGYPTI* UNDER FIELD CONDITIONS: MECHANISMS OF A POTENTIAL CONTROL TOOL

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Recent studies show that the behaviour of adult mosquitoes can be exploited for the dissemination of insecticides to aquatic habitats. This requires further characterization and optimization if it is to be widely adopted. A "lure and disseminate" device was designed that 1) contaminated wild mosquito populations with a potent pupacide and 2) released those mosquitoes to auto-disseminate pupacide to larval habitats. These dissemination tools were deployed in the field in Iquitos, Peru. Using coloured markers, the patterns and mechanics of dissemination between lures and sentinel oviposition habitats were examined. The potential contamination of the adult population was high and mark-recapture data revealed an even distribution of contaminated mosquitoes among sentinel aquatic habitats and a high frequency of contamination events at those habitats (ca 1 event every 4 days). Male mosquitoes, and other mosquito genera (particularly *Culex* spp) contributed to the dissemination process. The coloured markers were replaced with finely-milled pyriproxyfen (PPF) granules, and the impact of its subsequent dissemination by dispersing mosquitoes was assessed. Variation in juvenile mortality between aquatic habitats and trials was large (0-100%) but when all trials were averaged, 85% of sentinel larvae failed to develop. More than 75% of deaths occurred at the pupal stage. Affected aquatic habitats retained their pupacidal impact for 4 days after the contaminating devices had been removed from the trial site. The exposure of adult females to PPF using these dissemination tools also affected their reproductive potential. Only 46% of eggs laid at sentinel oviposition sites hatched. In contrast, 97% of eggs laid during control periods eclosed. These field results provide an essential understanding of the factors driving the remarkable efficacy of the auto-dissemination technique. These include 1) potency of the pupacide, 2) precise targeting of the insecticide by the mosquito, 3) amplification in coverage between the contaminating tools and the aquatic habitat, 4) persistence of the pupacide and 5) pupacides are unaffected by density dependent processes in the aquatic environment. The auto-dissemination technique, demonstrated here using a standardized contamination tool, a WHO-recommended pupacide, and a naturally-occurring mosquito population has enormous potential.

1465

CHARACTERIZATION OF STRAIN-SPECIFIC EFFECTS ON TRANSMISSION AND MAINTENANCE OF WEST NILE VIRUS

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West Nile virus (WNV; *Flaviviridae, Flavivirus*) cases in New York State (NYS), as well as nationwide, were historically high in 2012. In addition, maximum likelihood estimates based on testing of NYS mosquito pools demonstrated the highest prevalence in *Culex* mosquitoes since the introduction of WNV to NYS, with approximately 7.5 WNV-positive *Culex* per 1000 tested. Although environmental factors are likely important in driving epidemiological shifts, the role of both WNV consensus and intrahost genetic variation in governing temporal shifts in vectorial capacity has not been adequately assessed. In addition, variation in the capacity for vertical transmission and, therefore, overwintering success, has not been evaluated. Previous studies demonstrate that WNV effects on *Culex* life-history traits are strain-specific, establishing the need to evaluate factors beyond vector competence, including strain virulence in mosquitoes and alteration to both bloodfeeding and reproductive patterns, in order to accurately measure strain-specific differences in transmissibility and maintenance. To begin to evaluate these relationships, we used deep-sequencing to genetically characterize WNV strains isolated from *Culex* pools in Suffolk County, NY in both 2005 and 2012, representing low and high activity years, respectively, and performed subsequent phenotypic analyses including quantifying vector competence, life-history traits following exposure, and vertical transmission. Genetic analyses suggest consensus substitution rates of approximately 6 X 10^{-4}/base/year, which is comparable to what has been measured in previous studies, yet identification of single nucleotide polymorphisms (SNPs) demonstrate substantial differences in mutant swarm breadth between isolates, with 19 minority SNPs identified in a 3kb region of the WNV 2005 genome, relative to 9 for the WNV 2012 isolate. Preliminary studies also demonstrate differences in infectivity in *Cx. pipiens*; and initial assessment of life-history traits following exposure suggests potentially important strain-specific effects. Taken together, these data begin to inform our understanding of the relationship between WNV genetic variation and temporal fluctuation in WNV activity.

1466

NS3-249 AMINO ACID SUBSTITUTIONS ALTER AVIAN PATHOGENESIS OF BOTH LINEAGE 1 AND 2 WEST NILE VIRUSES

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Previous studies using selection modeling and experimental avian inoculations identified a single West Nile viral genetic *loci* (NS3-249) of lineage 1 WNVs to be under the effect of positive selection and to be a virulence determinant (NS3-249P) associated with increased replicative capacity and virulence in American crows (AMCRs), a sentinel species utilized in North America to track the spread of the virus. Although a lineage 2 virus was isolated from a moribund goshawk in Hungary in 2004, viruses from this lineage have not been associated with significant avian mortality. All genetically characterized lineage 2 viruses have been identified to have a His at the AMCR virulence locus, NS3-249; however, WNV isolates made from a large lineage 2 WNV outbreak in Greece in 2010 were identified to have a Pro at this site. A WNV infectious cDNA of a South African strain isolated in 1989 was subsequently generated and an NS3-H249P mutation incorporated to assess the potential modulatory effect of this locus in an alternative WNV lineage. Inoculation of AMCRs with the parental South African or cDNA clone-derived virus demonstrated mean peak viremias of 7-7.5 $\log_{10}$ PFU/mL sera and exhibited approximately 30% mortality. In contrast, the NS3-H249P lineage 2 mutant virus demonstrated 100% mortality in AMCRs with an approximate 100-fold higher mean peak viremia (9.6 $\log_{10}$ PFU/mL sera), indicating the potential importance of this specific genetic alteration within the lineage 2 genome for eliciting high replicative capacity in avian hosts. These results confirm the vital role of this locus for avian virulence potential and indicate the selective advantage of different NS3-249 residues for increased avian replication within both lineage 1 and lineage 2 WNV genetic backbones.

1467

SEQUENCE AND PHENOTYPIC ANALYSES OF 2012 WEST NILE VIRUS ISOLATES FROM TEXAS FAIL TO ASSOCIATE VIRAL GENETIC FACTORS WITH OUTBREAK MAGNITUDE

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In 2012, the U.S. experienced the largest outbreak of WNV human encephalitis since 2003. In order to determine whether the increase in WNV transmission in 2012 could have been due to recent sequence changes in the WNV genome, we sequenced 17 full-length isolates made from mosquito pools in Texas in 2012 and compared them to isolates from previous years. We found a similar amount of divergence in the 2012 Texas isolates compared to isolates from previous years, with most of the genome evolving under purifying selection and genetic drift. Further, we compared isolates from Dallas County, that exhibited a 2012 incidence rate of 16 WNV cases per 100,000 population, to isolates from Montgomery County, with a 2012 incidence of 3 WNV cases per 100,000 population. While genetic differences did exist between Dallas and Montgomery County viral populations, weak evidence supports genetic population subdivision or adaptive changes in the Texas isolates. Finally, *in vitro* growth rates of Dallas and Montgomery County WNV isolates with the aforementioned genetic differences were assessed in mammalian and mosquito cells. Results demonstrated that isolates with variable amino acids exhibited indistinguishable replication profiles compared to one another or to the NY99 strain, indicating that these 2012 WNV genetic differences did not afford an *in vitro* replication advantage. Together, these data do not support genetic viral adaptation as an explanation for increased WNV incidence in 2012.

1468

SMALL RNA RESPONSE OF *CULEX QUINQUEFASCIATUS* TO WEST NILE VIRUS INFECTION: RELATIONSHIP TO VECTOR COMPETENCE

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Culex mosquitoes are among the most important vectors of animal viruses worldwide. These include West Nile virus, Japanese Encephalitis virus, Rift valley fever virus and others. However, our understanding of the molecular events that influence their ability to transmit pathogens (vector competence) is incomplete. Variation in vector competence occurs between individual mosquitoes, between populations of the same species, and between taxonomically distinct species. When exposed to the same virus-containing bloodmeal, some mosquitoes fail to become infected, others become infected but limit virus replication and dissemination, while others develop disseminated infection and ultimately transmit virus. Vector competence has been shown through several studies to be a quantitative trait under the control of several genes and other factors. RNAi is widely

regarded as the most important antiviral pathway in mosquitoes, but its role in shaping mosquito vector competence is poorly understood. Moreover it is not clear how RNAi influences vector competence in non-transgenic "wild-type" vector mosquitoes. Therefore, we sought to characterize the mosquito RNAi response to WNV infection and determine its influence on vector competence using colonized *Cx. quinquefasciatus* mosquitoes. Mosquitoes were exposed to WNV in an artificial bloodmeal and held for various extrinsic incubation periods. Small RNA (sRNA) profiles were obtained using next-generation sequencing. To characterize the early sRNA responses to WNV, midguts were removed from mosquitoes 12 and 24 hours after feeding and sRNAs mapped to the WNV genome. To assess the relationship between sRNA responses and virus dissemination from the midgut (a prerequisite for virus transmission), midguts and legs were removed from mosquitoes at 7 and 14 days post feeding. sRNA responses from mosquitoes that permitted WNV dissemination from the midgut into peripheral tissues were compared to those with WNV limited to the midgut. Overall, these studies will characterize the sRNA responses of mosquitoes to WNV infection and determine the extent to which RNAi influences vector competence in this system.

1469

EVALUATION OF CHIMERIC JAPANESE ENCEPHALITIS VIRUS/ DENGUE VIRUS TYPE 4 VACCINE CANDIDATES IN MICE

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Japanese encephalitis virus (JEV) is a leading cause of viral encephalitis worldwide and vaccination is one of the most effective ways to prevent disease. A suitable live attenuated JEV vaccine could be formulated with a live attenuated tetravalent dengue vaccine for the control of these viruses in endemic areas. Toward this goal, we previously generated chimeric vaccine candidates by replacing the precursor membrane (prM) and envelope (E) structural genes of dengue virus type 4 (DEN4) or attenuated DEN4Δ30 with those of JEV India/78. These first generation JEV/DEN4 chimeric viruses were attenuated for neurovirulence and neuroinvasiveness in weanling mice compared to the wild-type JEV parent, which warranted their further development as vaccine candidates. Adventitious mutations in E, NS3 and NS4B proteins that arose during adaptation of first generation chimeric viruses for replication in Vero cells were engineered into a second generation of JEV/DEN4 chimeric viruses. Novel 3'UTR deletions, similar to those found in DEN4Δ30, were also introduced. Sequencing revealed that chimeric viruses lacking engineered Vero cell adaptive E protein mutations acquired adventitious mutations. This suggests that at least one adaptive E protein mutation is required for genetic stability of JEV/DEN4 chimeric viruses propagated in Vero cells. The second-generation chimeric viruses were attenuated for neurovirulence and neuroinvasiveness in weanling mice. They were also significantly more attenuated for neurovirulence in suckling mice than the wild-type JEV parent and the JEV SA14-14-2 live-attenuated vaccine strain, based on LD50 values and survival times. Deletions in the 3'UTR also increased attenuation for suckling mice. By contrast, a single E protein mutation that is shared by JEV SA14-14-2 significantly increased neurovirulence in suckling mice and replication in Vero cells for one chimeric virus. We are currently evaluating these chimeric vaccine candidates in mice for immunogenicity and protection from challenge with wild-type JEV.

1470

ESTIMATING THE BURDEN OF YELLOW FEVER IN AFRICA

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Yellow fever is a vector-borne disease affecting humans and non-human primates in tropical areas of Africa and South America. While eradication is not possible due to the wildlife reservoir, large scale vaccination activities in Africa in the 1940s to 1960s reduced yellow fever incidence for several decades. However, after a period of low vaccination coverage, yellow fever has resurged in the continent. Since 2006 there has been substantial funding for preventive mass vaccination campaigns for yellow fever in the most affected countries in Africa to curb the rising burden and control future outbreaks. Generalised linear regression models were fitted to a dataset of the locations of yellow fever outbreaks in the last 25 years to estimate the probability of outbreak reports across the endemic zone. Environmental variables and indicators of surveillance quality in the affected countries were used as covariates. By comparing probabilities of outbreak reports estimated in the regression with the force of infection estimated for a limited set of locations for which serological surveys were available, the detection probability per case and the force of infection were estimated across the endemic zone. The yellow fever burden in Africa was estimated for the year 2013 as 130,000 (95% CI 84,000 - 170,000) severe cases including 44,000 (95% CI 29,000 - 60,000) deaths, taking into account the current level of vaccination coverage. The recent mass vaccination campaigns are estimated to have reduced this burden by 27% (95% CI 23 - 30%) across the region, achieving up to 82% reduction in countries targeted by these campaigns. With the estimation method presented here, spatial estimates of transmission intensity can be combined with vaccination coverage levels to evaluate the impact of past or proposed vaccination campaigns, thereby helping to allocate resources efficiently for yellow fever control. *Expert Committee: Donald Burke, Fernando De La Hoz, Bryan Grenfell, Peter Hansen, Raymond Hutubessy, Rosamund Lewis, William Perea, Olivier Ronveaux, Erin Staples, Sergio Yactayo.

1471

ISOLATION AND CHARACTERIZATION OF PARAISO ESCONDIDO VIRUS: A NEW FLAVIVIRUS *IN LUTZOMYIA (PSATHYROMYIA) ABONNENCI* SANDFLIES FROM ECUADOR

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Flaviviruses consist of mosquito-borne, tick-borne, insect-only and non-vectored viruses. Sandflies are not recognized as principal vectors of flaviviruses. We report here the discovery (detection, isolation and full-length sequence) of a novel flavivirus in *Lutzomyia (Psathyromyia) abonnenci* that was provisionally named Paraiso Escondido virus. Twenty six pools of *Lutzomyia* flies were screened for the presence of flaviviruses. One pool of female (neither gravid nor engorged*) Lutzomyia (Psathyromyia) abonnenci* was found to contain flavivirus RNA through real time RT-PCR assay targeting all flaviviruses, as previously reported. Assuming that one sandfly only was infected in the pool, quantitative real-time PCR estimated that > 1012 genome copies were in the infected insect individual. Virus isolation was obtained in C6/36 cells. The complete genome was sequenced using next generation sequencing technology based on Ion-torrent PGM. The genome consisted of 10,760 nucleotides encoding 3441 AA with 5'- and 3'-UTR of 119 and 316 nts,

respectively. A series of cysteine residues and potential glycosylation sites were identified. The enzymatic domains (serine-protease, helicase/NTPase, methyltransferase and RNA-dependent RNA polymerase) of Paraiso Escondido virus were found to be highly conserved in comparison with other flaviviruses. The putative cleavage sites of the polyprotein were identified and found substantially different from those of other flaviviruses. The AA distances observed ranged 53-85%, 40-72%, 35-56% with envelope, NS3 and NS5 proteins. Phylogenetic analyses based on amino acid alignments showed that Paraiso Escondido virus clusterized together with *Aedes*-borne flaviviruses although it is clearly distinct from other known flaviviruses. In the New world, *Lutzomyia* sandflies are the vectors of viruses (vesicular stomatitis virus, Orbivirus, Punta Toro virus), parasites (leishmaniasis) and bacteria (bartonellosis). Therefore they should be considered as possible vectors of viruses of potential medical and veterinary importance. Further investigations are on-going to determine whether Paraiso Escondiso virus is capable to infect vertebrates and humans.

1472

INTEGRATED, COMMUNITY-BASED SURVEYS OF INTESTINAL PARASITIC INFECTIONS WITH TRACHOMA IMPACT ASSESSMENTS IN AMHARA NATIONAL REGIONAL STATE, ETHIOPIA

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In the Amhara National Regional state of Ethiopia we integrated assessment of intestinal parasitic infections into large-scale trachoma impact surveys to establish baseline prevalence upon which to monitor the impact of integrated control measures of improved hygiene, water, sanitation, and preventive chemotherapy. Both trachoma and intestinal parasites (*Schistosoma mansoni*, soil-transmitted helminths, and intestinal protozoa) were assessed in systematically selected clusters from a geographic listing of communities by district. One child aged 6-15 years per household in selected clusters was randomly selected to provide a stool sample of which about 1 g was preserved in sodium acetate-acetic acid-formalin, processed using formol-ether concentration and examined under a microscope by experienced laboratory technicians. A total of 6,732 stool specimens were collected from 368 communities. The prevalence of *S. mansoni* was 6.6% (range by district 0-40.9%), but prevalence in 55 communities was ≥10%. The overall prevalence of any soil-transmitted helminth infection was 22.5% (range by district 3.0-77.7%). Approximately 3 in 4 children were infected with at least one intestinal protozoa. The prevalence of Giardia intestinalis was 18.9% (range by district 5.4-41.0%) and Entamoeba histolytica/E. dispar was 12.5% (range by district 2.9-22.5%). Associations between soil-transmitted helminth infections and community-level indicators of hygiene, water, and sanitation were explored. According to World Health Organization guidelines, preventive chemotherapy targeted to school-aged children is warranted for the control of schistosomiasis in 10 and for the control of soil-transmitted helminths in 39 out of 59 districts. Integration of deworming with mass distribution of antibiotics for trachoma might further expand health benefits to co-endemic communities. Integrating assessment of intestinal parasitic infections with community-based trachoma prevalence surveys may be a feasible method for evaluating impact of neglected tropical disease control programs.

1473

INTEGRATED SCHOOL-BASED SURVEILLANCE FOR SOIL-TRANSMITTED HELMINTH INFECTIONS AND FOR LYMPHATIC FILARIASIS IN GAMPAHA DISTRICT, SRI LANKA

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The Sri Lankan Anti-Filariasis Campaign (AFC) conducted 5 rounds of annual mass drug administration (MDA) with albendazole and DEC in 2002-2006 in 8 districts that were endemic for lymphatic filariasis (LF) (target population approximately 10 million). AFC conducted transmission assessment surveys (TAS) in 2012, about 6 years after the last round of MDA. This study explored the practicality of integrating surveillance for soil transmitted helminth (STH) infections with TAS for LF in Gampaha district (population 2.3 million). The district was divided into two Evaluation Units (EUs), coastal and inland. Each TAS tested 1st and 2nd grade school children drawn from 30 randomly selected schools (N=1,462 inland, 1,642 coastal). Tests included the ICT card test for filarial antigenemia (performed by AFC personnel) and the Kato-Katz test for detection of STH ova (performed by university personnel). ICT rates were 0% and 0.1% (0.01-0.3% CI) in the inland and coastal EUs, respectively. These results suggest that LF transmission rates are very low in Gampaha District. The STH survey was conducted at the same time as the TAS in the inland EU (955 stools from 1,211 children) and several weeks after the TAS in the coastal EU (927 stools from 1,586 children). STH infection rates and stool sample participation rates were 0.8% and 79% in the inland EU and 2.8% and 58% in the coastal EU. Most of the STH infections detected were low-intensity*Trichuris* (present in 73% of positive stools). The low STH rates are probably due to the country's national school deworming program (mebendazole in grades 1, 4, and 7) and relatively good sanitation in Gampaha district. The cost for STH testing was approximately $5,000 per EU. These results suggest that it is feasible for national NTD programs to integrate school based surveillance for STH and LF. Further work is needed to streamline procedures and to determine optimal sampling strategies for STH surveys, because these may not require as many samples or sampling sites as TAS.

1474

QUANTIFYING THE QUALITY OF SURVEY DATA FOR THEIR USE IN THE DESIGN OF SOIL TRANSMITTED HELMINTH AND SCHISTOSOMIASIS CONTROL PROGRAMS

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Generous medication donations and increasing political commitment has led to the launch of numerous national neglected tropical diseases (NTD) control programmes. The first step in developing these programmes is often country-wide mapping of disease, which might be unnecessary if data from previously conducted studies exist. Thus, large scale surveys would waste resources and cause unnecessary delays for medication distribution. As the quality of previously collected data can vary in terms of study design and data collection, we developed guidelines for the use of available soil transmitted helminth (STH) and schistosomiasis survey results to support programme implementers during the process of programme design. These guidelines allow identifying areas where sufficient information already exists and others that should be prioritised for surveys. The approach is based on three steps: i) the identification of ecological

zones within the country, ii) the identification and classification of available surveys and iii) the classification of each ecological zone based on characteristics of individual surveys. The country is divided into ecological zones based on environmental data and a systematic literature research is performed to capture all relevant studies. The quality of the identified surveys is assessed based on the following characteristics: We considered the time since surveys, as well as expected substantial changes in transmission. Additionally, the representativeness of the study population is taken into account, as well as the sampling design and sample size. The accuracy of diagnostic methods is graded based on published comparative studies of diagnostic tools. Furthermore, the level of available information is assessed in terms of geographic information (precise locations of surveys vs. district or province summary estimates) and infection information (species specific prevalence vs. STH summary estimates). According to the coverage and quality of identified surveys, each ecological zone is classified independently into five groups i) mapped as recommended, ii) mapped, iii) mapped with low quality data, iv) mapped with questionable data, and v) not mapped. Based on the grading of ecological zones, we further provide recommendations for the design of national surveys. Finally, we demonstrate the application of the proposed guidelines on the example of Kenya and discuss their potential constraints.

1475

TREATMENT COVERAGE OF INTEGRATED MASS DRUG ADMINISTRATION FOR NEGLECTED TROPICAL DISEASES IN TOGO

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Since 2009, the country of Togo has implemented a program for the integrated control of neglected tropical diseases. Under this program, onchocerciasis, schistosomiasis, and soil transmitted helminths are targeted using community-based distribution of ivermectin (IVM), praziquantel (PZQ), and albendazole (ALB). Drugs are given to selected populations based on local prevalence of each disease. A nationwide integrated mass drug administration (MDA) was conducted in July 2012; while reported treatment coverage was high, integrated MDA campaigns are logistically complex and reported coverage may not reflect the actual coverage achieved. In November 2012, Togo conducted a survey to validate coverage for all three diseases. Four cluster surveys were conducted, one in each of three geographically disparate districts, and a fourth in areas with high prevalence of onchocerciasis. In each district 30 to 45 villages were selected with probability proportional to size. Ten houses were selected in each village and all household members were asked about receipt of drugs in July 2012. Each household head answered questions about knowledge of the diseases. In the four clusters a total of 9511 persons in 1187 households were interviewed. Coverage varied by district: 74-84% of the population received IVM, 80-94% of school-age children (SAC) received PZQ, and 84-94% of SAC received ALB. Measured coverage for PZQ and ALB exceeded WHO targets; coverage for IVM was below WHO target in two districts. In one district measured coverage was lower than reported for all drugs ($P<0.05$), and in two others measured coverage was lower than reported for ALB. Coverage validation surveys are an important part of program evaluation. The sampling for this survey was challenging and novel since the targeted diseases and populations vary by village and no clear guidelines exist for sampling in such a complex distribution scheme. Results from this study will be used to refine MDA training and supervision, and will also contribute to decisions regarding control and/or elimination strategies for these diseases.

1476

"COORDINATED" MAPPING FOR NEGLECTED TROPICAL DISEASES IN COTE D'IVOIRE

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Where geographic overlap among NTD distribution exists, coordinated mapping could result in significant resource savings. There are few data on the geographic distribution of NTDs in post-conflict Cote D'Ivoire. To guide intervention, by the recently established national Lymphatic Filariasis, Schistosomiasis and Soil Transmitted Helminth Control Programme, a coordinated prevalence survey for schistosomiasis, soil-transmitted helminth (STH) infection and lymphatic filariasis (LF) was conducted. The aim was to design a resource efficient protocol to establish which communities required mass drug administration (MDA), according to World Health Organization thresholds. The sampling frame was the health district with a total of eight health districts sampled. Within each health district 20 communities were surveyed for schistosomiasis and STH, sampling 50 school-age children per village. Among the 20 communities selected, two were also surveyed for LF with 100 adults sampled in each. In total 8,000 school-aged children were tested for both urinary and intestinal schistosomiasis and STH. A further 1600 adults were tested for circulating *Wuchereria bancrofti* antigen using immunochromatographic card tests (ICT). Preliminary analysis has shown prevalence of *Schistosoma haematobium* and *S. mansoni* ranged from 2-69% and from 0-76%, respectively. The main STH species was hookworm, ranging from 2-41% by village. LF and cost-analysis results are under-going preliminary analysis. This was the first attempt at using a coordinated survey design for this group of infections in Cote D'Ivoire. The approach proved practical and the results show that only a few areas need to be targeted with MDA, thus confirming the importance of detailed mapping for cost-effective control.

1477

BARRIERS TO COMPLIANCE WITH MASS DRUG ADMINISTRATION FOR NTD CONTROL PROGRAMS

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Compliance with mass drug distribution is a determinant of success for national neglected tropical diseases (NTD) control programs. Persistent non-compliance can maintain diseases transmission as a reservoir for potential re-infection. Out-of school children, women of reproductive age and hard-to-reach remote populations might repeatedly miss opportunities for mass drug distribution (MDA); creating systematic non-compliant groups for preventive chemotherapy. An analysis of semi-annual reports and post MDA coverage surveys from the USAID funded NTD control programs and other NTD projects was conducted to identify barriers to adhering to MDA treatment schedules and keys factors amenable to corrective measures. The review identified several groups of individuals including institutionalized people, non-enrolled school age children, and people from high socio-economic status as persistently non-compliant during MDAs. Also identified were 47% of women excluded from MDA due to pregnancy or nursing of babies under 1 month of age, and who later qualify for treatment but did not attend consecutive rounds of annual MDAs. Large sections of urban populations in endemic settings remain consistently untreated because they present specific challenges in terms of acceptability of drugs distributed by non health professionals. Other reasons for recurrent non participation in mass campaigns include: 1) fear of adverse event, 2) non perceived benefit, and 3) lack of disease awareness. The authors also explore campaign fatigue resulting from the long standing drug distribution programs. Strategies to overcome barriers and to increase compliance involve refinement of pre-MDA plans, comprehensive registration of all eligible populations, intensive IEC campaigns, implementation of flexible distribution mechanisms, and routine facility-based post campaign drug administration. The authors

recommend adapting MDA to the local environment and making use of platforms and local opportunities to increase NTD programs visibility and drug coverage.

1478

MONITORING AND EVALUATING INTEGRATED NTD CONTROL: FIVE-YEAR IMPACT OF TREATMENT ON INFECTION IN NIGER AND BURKINA FASO

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Monitoring and evaluation (M&E) is an essential element of national NTD control that guides a programme on how to strengthen its approach. Standard epidemiological monitoring methods, used to measure the impact of treatment through annual parasitological examinations at school sentinel sites, are being employed across all SCI countries. The findings from two countries are presented here. A longitudinal cohort of 718 and 2405 children aged 7-12 years in Niger and Burkina Faso, respectively, were recruited at baseline (2004) and parasitological examinations carried out at yearly intervals before and after large-scale treatment for schistosomiasis and STH. Preventative chemotherapy (PCT) was integrated against five NTDs (lymphatic filariasis, schistosomiasis, STH, onchocerciasis and trachoma) in 2007. In order to monitor the impact of combined mass drug administration, integrated (schistosomiasis, STH, and trachoma) sentinel schools were added in 2008. Data from the longitudinal cohort demonstrated that a significant decrease in the odds of detectable trachoma, as well as *Schistosoma haematobium* infection, was found at follow-up two years post-baseline. Children who benefited most from anthelmintic treatment, in terms of increased haemoglobin concentrations, were those who had presence of anaemia and highly positive microhaematuria scores at baseline. This study demonstrates that chemotherapy can have a substantial impact on both S.*haematobium* and trachoma infection, and its associated morbidity in children, even after integrating PCT for several NTDs. These are the first known integrated sentinel sites to examine all three NTDs, the results of which will demonstrate whether the presence of co-infection affects the impact of treatment as well as the cost-efficiency of combining M&E for multiple infections.

1479

THE UTILITY OF DIAGNOSTIC TESTS FOR TYPHOID FEVER AT CHITTAGONG MEDICAL COLLEGE HOSPITAL (CMCH), CHITTAGONG, BANGLADESH

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Typhoid fever (TF) is commonly diagnosed in febrile patients in Bangladesh but confirmatory tests are unsatisfactory. We evaluated BactAlert® blood cultures; an in-house real time PCR; and rapid antibody diagnostic tests (RDT) for TF in febrile adults and children admitted to CMCH. The RDTs were Life Assay Test-it™ Typhoid IgM lateral flow assay detecting IgM antibodies against *S. enterica* Typhi (ST) O antigen; CTKBiotech Onsite Typhoid IgG/IgM Combo Rapid-test cassette lateral flow assay detecting IgG and IgM antibodies against ST O and H antigens; and SD Bioline line assay for IgG and IgM antibodies against ST proteins. Background antibody levels were studied 40 local adult healthy controls: Life Assay RDT was positive at 1+ in 30 controls but at > 1+ in only one control; the CTK and SD Bioline kits were negative in all controls. We studied 303 febrile patients admitted to CMCH with a median (IQR) age of 13 (5-31) years and median (IQR) duration of illness before admission of 5 (2-8) days. TF was diagnosed in 57 (18.8%): 19 positive by blood culture with ST (3 also blood PCR positive); 20 blood culture negative but PCR positive in blood (15), urine (4) and faeces (2); and 18 blood culture and PCR negative but with a compatible clinical syndrome. Of the 246 patients without TF, 13 had a significant positive blood culture with other bacteria. We calculated the sensitivity, specificity, positive and negative predictive values of the three RDTs comparing those patients who had blood culture and/or PCR confirmed typhoid (n=39) with those without TF (n=246). For the Life Assay IgM LFA at a cut-off of ≥ 2+ the sensitivity, specificity, PPV and NPV were 36%, 89%, 35% and 90%; for CTK IgG/IgM assay the values were 54%, 74%, 25% and 91%; and the SD Bioline IgG/IgM assay values were 21%, 97%, 50% and 89%. The performance characteristics of the RDTs were insufficient to be clinically useful. Although the addition of PCR to BC increased the number of laboratory confirmed cases, the evaluation of RDTs is still hampered by the lack of a gold standard for TF diagnosis.

1480

THE FORGOTTEN SCOURGE: MODELING DYNAMICS AND CONTROL OF ENDEMIC TYPHOID IN KATHMANDU, NEPAL

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Typhoid is a paradoxically widespread yet neglected disease. Recent estimates place the global typhoid burden from 13.5 – 26.9 million cases and 190,000 – 216,000 deaths annually, which provides a motivation to better understand typhoid dynamics. We developed an age-structured, compartmental model that was representative of the pathogen's natural history and human immune response to the infection. We fit the model to incidence data from 1997-2011 collected from Patan Hospital in Kathmandu, Nepal in order to estimate unknown model parameters. The model assumed indirect transmission was a function of rainfall and reproduced the timing of annual peaks very well, but failed to account for an upward trend in cases that began in 2000. An adjusted form of the model that incorporated antibiotic resistance reproduced both the timing and magnitude of the epidemic peaks over the entire dataset. This lends support to the hypothesis that increased use of fluoroquinolones drove the clonal expansion of the H58 haplotype, which confers nalidixic acid resistance through a mutation in DNA gyrase *gyr*A. The inclusion of migrant male workers entering Kathmandu with no previous typhoid exposure helped explain an observed shift in the age and gender distribution of cases, suggesting migration patterns partially underlie typhoid dynamics. The calibrated model estimated the basic reproductive number (R_0) to be ~4.5 in this setting. School-based vaccination was predicted to produce indirect protection and decreased typhoid incidence in the short-run, but the incidence rate is expected to rebound in about 5 years, shortly after vaccine-induced immunity wanes. As the Nepali government begins to implement school-based vaccination, government and public health authorities must recognize the limitations and potential adverse effects of a one-time vaccination campaign. Water and sanitation improvements will be critical to typhoid elimination.

1481

BURDEN OF LABORATORY-CONFIRMED SHIGELLOSIS INFECTIONS IN GUATEMALA 2007-2012: RESULTS FROM A POPULATION-BASED SURVEILLANCE SYSTEM

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In Guatemala, diarrhea is the second most common cause of morbidity and mortality in children <5 years of age. The proportion of diarrheal disease caused by Shigella *sp.* remains unknown. Using data collected from the two hospitals and 10 clinics in active population-based surveillance sites in Quetzaltenango (Average High Temperature: 22°C) and Santa Rosa (Average High Temperature: 31°C) Departments, we describe the epidemiology and antimicrobial susceptibility patterns of culture-confirmed Shigella infections. Clinical, epidemiological, and laboratory data were collected on patients presenting with acute diarrhea (≥3 loose stools in 24 hours), from June 2007 - August 2012. Of 5,399 stool specimens collected from patients who met the case definition, 261 (4.8%) yielded *Shigella sp.*. Most were *S. flexneri* (59.2%) followed by *S. sonnei* (35.6%). Most (51%) infections occurred from May to August, during the rainfall season. During the 5 years, the incidence of laboratory-confirmed infections varied from 5.0 to 24.1 per 100,000 in Santa Rosa and 0.31 to 6.2 in Quetzaltenango. Most (57.9%) cases occurred in children <5 years of age; incidence in this age group were 91.9 per 100,000 in Santa Rosa and 31.1 in Quetzaltenango. Thirty (12%) patients were hospitalized, including 6 who were admitted to the intensive care unit. Three patients experienced convulsions, 5 bloody diarrhea, and 17 vomiting; there was 1 death. Over half (56%) of cases were treated with oral rehydration solution within three days of enrollment and 76% of hospitalized cases received intravenous fluids. Antimicrobial susceptibilities were tested for 260 isolates; 238 (96%) were resistant to tetracycline, 210 (83%) to trimethoprim-sulfa, and 141 (61%) to ampicillin. No isolates were resistant to the quinolone antibiotics tested. Shigella is an important cause of bacterial diarrhea in children <5 years of age in Santa Rosa and Quetzaltenango. Though limitations exist in the surveillance reporting, the reported incidence is likely an underestimate and highlight the importance of optimizing treatment regimens. Identification of specific risk factors for infection may allow for targeted prevention interventions.

1482

EPIDEMIOLOGY OF *STREPTOCOCCUS SUIS* INFECTION IN THE SOUTH OF VIETNAM

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Human *Streptococcus suis* infection is an emerging zoonostic disease in Southeast Asian countries. It is the most common pathogen caused bacterial meningitis at two referral hospitals for infectious diseases in Ha Noi and Ho Chi Minh City, Vietnam. To date, there has not been any information related to this pathogen in other hospitals as well as the incidence rate of this disease in Vietnam. A prospective hospital-based descriptive surveillance study was conducted from 08/2007 to 04/2010 at thirteen hospitals in central and southern Vietnam, including one district hospital, ten provincial hospitals and two central referral hospitals. Patients were recruited if they met all of the following inclusion criteria: at least one month of age; fever ≥380C (axillary); at least one of the following symptoms or signs: headache, neck stiffness, altered consciousness and focal neurological signs; and a cerebrospinal fluid (CSF) sample taken. *S. suis* was confirmed in CSF and blood samples by using classical microbiology and molecular diagnostics. A total of 1740 patients suspected central nervous system infection were recruited, in which *S. suis* was confirmed in 149 cases. *S. suis* was not found in children but it was reported as the most common pathogen of adult bacterial meningitis (149/302, 49%) in most of provincial hospitals. Overall incidence rate was 0.57/100,000 adult person-years. Incidence rate increased significantly with incremental age group. The ratio between males and females was 4:1. Pig exposures, such as breeding pigs at home, slaughtering pigs and eating raw or undercooked blood/organs, were described in 59/149 (40%) patients. Bacterial meningitis, the most common manifestation of *S. suis* infection, was responsible for 146/149 (98%) cases, and septic shock, the most serious manifestation, was reported in 3/149 cases (2%) and the overall case fatality rate (CFR) was 8% (12/149). While seasonality of *S. suis* meningitis was reported at Hue Central hospital (a tertiary hospital of the North Central of Vietnam), of which the peak month was estimated as July ($p<0.001$), it was not observed in other southern provinces ($p=0.31$). In conclusion, this study indicated that *S. suis* serotype 2 meningitis is endemic in Vietnam. Health education program on prevention should be applied to high risk groups to reduce the loss of health and economics of community.

1483

EPIDEMIOLOGY AND RISK FACTORS OF SEROGROUP W135 MENINGOCOCCAL DISEASE OUTBREAK IN THE GAMBIA, FEBRUARY-JUNE 2012

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In the African meningitis belt, meningococcal disease is endemic with regular outbreaks, mostly (80%) due to *Neisseria meningitidis (*Nm*)* serogroup A. In 2002-2003, a large epidemic of NmW135 occurred in this region, but not in The Gambia, where the last cases were reported in 1995. In 2012, another NmW135 epidemic occurred in the meningitis belt, including The Gambia. Between February and June 2012, the Gambian Ministry of Health and the Medical Research Council (MRC) Unit, The Gambia, investigated this outbreak in the Central (CRR) and Upper (URR) River Regions. Suspected cases were identified in Bansang Hospital, CRR, and Basse Health Centre, URR, and by visiting NmW135 cases' households. A suspected case was defined as any patient with history of acute fever and any of the following: altered consciousness, unable to feed, neck stiffness, convulsion, petechial rash or bulging anterior fontanel. Cerebrospinal fluid and blood samples were collected from hospitalized cases to identify the pathogens by culture and latex test. A confirmed case was a suspected case in which NmW135 was identified by culture and/or an antigen-specific test. A matched case-control (1:1) study was carried out. Healthy controls were matched with confirmed cases by age and village. We identified 469 suspected cases of which 114 were confirmed for NmW135. Most (65%) of them were in children <5 years old. The overall attack rate was 111/100,000 population but in children <5 years it was 5 times higher (485/100,000) than in older children and adults. The epidemic threshold (10 cases/100,000 population/week) exceeded in February and continued until April in all ages and until June in children <5 years. In the multivariate analysis, male gender (OR 1.9; 95% CI 1.0-3.7), contact with cases (OR 4.8; 95% CI 1.3-17.8), difficult breathing (OR 6.8; 95% CI 1.4-33.4) and itchy eyes (OR 4.4; 95% CI 1.3-14.4) were significantly associated with NmW135 cases. Enhanced surveillance of meningitis and multi-serogroup conjugate vaccine are recommended for the control and prevention of meningococcal epidemics.

1484

RODENT CONTROL PROGRAM AND LEPTOSPIROSIS PREVENTION IN SALVADOR, BRAZIL

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Effective interventions for leptospirosis have not been identified which can be feasibly implemented in urban slum communities. Rodent Control Programs (RCP) constitute the principal strategy to prevent leptospirosis in Brazilian cities. However, RCPs are expensive and their efficacy has not been evaluated. We evaluated the efficacy of a municipal RCP to decrease rat infestation and leptospirosis incidence in Salvador (2.6 million pop.). Residences of patients with laboratory confirmed leptospirosis (years 2005-10) were geolocated and used to define 11 areas (15% of the city), containing 2,078 blocks, of equal risk for leptospirosis. During a pre-epidemic season (January-April), households from selected blocks were surveyed for rat infestation and received rodenticide application. Efficacy of the RCP was evaluated after the intervention by assessing two outcomes: 1) rat infestation by surveying 10% of treated blocks, and 2) change on incidence of leptospirosis. Kilograms of applied rodenticide and proportion of treated houses per block were used as measures of treatment intensity. These intensity proxies were used to build two mathematical models and evaluate the risk ratio of incidence of leptospirosis between pre- (2005-08) and post-intervention (2009-10) periods. A total of 671 blocks were treated in 2009 and 1,129 blocks in 2010. Surveys identified rat infestation of 25% and 26% in 2009 and 2010, respectively. 92% of the infested households in 2009 had evidence of *Rattus norvegicus*. After intervention, rat infestation decreased from 25% to 7% ($p<0.001$) in 2009 and from 26% to 15% ($p<0.001$) in 2010. The incidence of leptospirosis in the study blocks was 32.2 and 11.3 per 100,000 pop. during the pre and post-intervention periods, respectively. Using the models, the predicted reduction in incidence after maximum rodent intervention, as measured by either completeness of block coverage or kg of rodenticide, had large confidence intervals limiting our ability to evaluate the efficacy of RCP. Our study indicates that a high proportion (>25%) of households are infested with *R. norvegicus*. The RCP was able to decrease rat infestation, but because severe leptospirosis cases are rare events, it was not possible to evaluate their efficacy to decrease incidence. Further evaluations, considering more frequent events, such as mild *Leptospira* infection, may be necessary to evaluate the effectiveness of these costly interventions.

1485

DEVELOPMENT OF DIAGNOSTIC AIDS TO DISCRIMINATE PARTIALLY TREATED BACTERIAL MENINGITIS (PTBM) FROM VIRAL MENINGITIS/ENCEPHALITIS (VM/EN)

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The diagnosis of ptBM is difficult. Discrimination of cases from those of VM/EN by clinical features alone is often impossible. We aimed to create a simple diagnostic aid for ptBM in adults on the basic laboratory features. We compared the laboratory features on admission of 374 adults at HTD, Vietnam who satisfied diagnostic criteria for ptBM (n=291) or VM/EN (n=83). Laboratory features independently predictive of ptBM were modelled by logistic regression according to Bayesian information criterion (BIC) and by classification- tree (C-T) method. Prognostic accuracy was summarized by sensitivity/ specificity / positive predictive value (PPV)/ negative predictive value (NPV). To assess potential over fitting of our models, all performance measures were bootstrap corrected for optimism. BIC defined three characteristics independently predictive of a diagnosis of ptBM from VM/EN: cerebrospinal fluid (CSF) neutrophil proportion (N%), CSF: blood glucose, and log2 (CSF lactate). Using these three predictors we developed a diagnostic nomogram. Our C-T constructed on two predictors (CSF lactate and CSF white cell count) which is more simple than nomogram but less sensitivity, specificity, PPV and NPV than those in BIC (0.979, 0.923, 0.978 and 0.929, and 0.984, 0.962, 0.988 and 0.950, respectivively). This study suggests that simple laboratory data can help in the diagnosis of adults with ptBM, particularly in setting with limited microbiological resources.

1486

VACCINATION WITH A GENETICALLY MODIFIED FILARIAL CYSTEINE PROTEASE INHIBITOR-2 PROTECTS GERBILS AGAINST *BRUGIA MALAYI* AND MICE AGAINST *ONCHOCERCA VOLVULUS* INFECTION

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Cysteine protease inhibitors or cystatins are reversible, tightly binding inhibitors of cysteine proteases. Filarial cysteine protease inhibitors have been ascribed to participate in worm's development as well as to contain immunomodulatory properties. They are hypothesized to play an important role in the establishment of infection by suppressing host immune responses, and therefore are good candidates for vaccine development. Expressed recombinant wild-type *B. malayi* cysteine protease inhibitor-2 (Bm-CPI-2) and *Onchocerca volvulus* cysteine protease inhibitor-2 (Ov-CPI-2) in *E. coli* showed strong inhibitory activity against Cathepsin L. Since the wild-type cystatin is a strong immune suppressor and therefore could inhibit host immune response upon immunization, the amino acid Asn at position 66 related to its asparaginyl endopeptidase inhibition activity was mutated to Lys66 in order to inactivate its immune suppressive activity and therefore enhance its protective immunity. DNAs encoding the Bm-CPI-2 or Ov-CPI-2 minus the signal peptides, with Asn66 mutated to Lys66 (Bm-CPI-2M, Ov-CPI-2M) were synthesized by GenScript and subsequently subcloned and expressed in the *E. coli* expression vector pET41a. Mongolian gerbils were immunized with 25 µg of the recombinant Bm-CPI-2M intraperitoneally with alum as the adjuvant three times, two weeks apart. The gerbils were challenged with infective L3 larvae subcutaneously and the parasites were recovered on day 42 post-infection. Vaccination with Bm-CPI-2M resulted in 48% reduction in worm burden in comparison to the Alum control group. Measurement of Bm-CPI-2M specific IgG by ELISA showed elevated levels of specific antibody response in the Bm-CPI-2M vaccinated gerbils. Vaccination of mice with the *O. volvulus* modified cystatin, Ov-CPI-2M, in alum also induced protection against larvae implanted subcutaneously within diffusion chambers, resulting in a 27% reduction in parasite survival. The immunized mice developed antigen-specific IgG responses. Our results confirm the CPI-2M vaccine-mediated protection obtained in the murine model of filariasis Litomosoides sigmodontis (Babayan et al. 2012), and extend it to filarial parasites of humans. In conclusion, the genetically modified filarial cysteine protease inhibitor-2 is a promising candidate for use in prophylactic vaccines against filariasis.

1488

EARLY DNA METHYLATION EVENTS IN UROGENITAL SCHISTOSOMIASIS AND THEIR IMPLICATIONS FOR INFLAMMATION-INDUCED BLADDER CANCER

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Urogenital schistosomiasis is linked to inflammation-associated bladder cancer. Inflammation-induced DNA methylation of tumor suppressor genes has been implicated in various forms of carcinogenesis. We hypothesized that some of these DNA methylation changes could be detected early after induction of experimental urogenital schistosomiasis. We combined our established mouse model of urogenital schistosomiasis with reduced representation bisulfite sequencing (genome-wide methylation analysis). Mice underwent sub-epithelial bladder injections of *Schistosoma haematobium* eggs or vehicle. Other mice received drinking water containing nitrosamine, an established urothelial carcinogen. After two weeks of exposure mice were sacrificed and their urothelia dissected from the detrusor and granuloma. DNA was extracted from each specimen and the restriction enzyme Msp1 used to cleave CpG islands. After bisulfite treatment samples were purified to a length of 175-225 bp and amplified by PCR. Next generation sequencing was performed with the Illumina Hi-Seq platform. The output was aligned with the UCSC M. Musculus genome v10 using Bismark software. Methylation analysis was performed with Methylkit and IGV. Egg-injected mice featured major alterations in their methylome (vs control mice) after two weeks of treatment. Bases with a depth of sequencing of less than 10 were excluded from analysis, and differential methylation was defined as a different of greater than 25% with a p-value of less than 0.05. 13,333 cytosines were hypermethylated and 6,244 were hypomethylated. Of these differentially methylated bases 1019 were found to be within 1000 base pairs of a transcription start site for a known gene. Six of these genes are part of the Wnt canonical pathway, which is related to cell proliferation. A CpG upstream of the Wnt Inhibitory Factor-1 gene, a gene silenced by hypermethylation in bladder tumors and other cancers, was methylated 54% of the time in egg-injected mice, 34% in nitrosamine-fed mice and 7% in control mice. This methylation event, along with our profiling of the DNA methylome of mice with experimental urogenital schistosomiasis, is the first of its kind, and may lead to an understanding of the sentinel events of urogenital schistosomiasis-associated bladder carcinogenesis.

1489

INFECTION WITH CARCINOGENIC LIVER FLUKE *OPISTHORCHIS VIVERRINI* MODIFIES INTESTINAL AND BILIARY MICROBIOME

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Opisthorchis viverrini is a fish-borne trematode endemic in East Asia. Following ingestion, the flukes locate to the biliary tract, where chronic infection frequently leads to cholangiocarcinoma (CCA). The precise mechanism(s) by which *O. viverrini* infection culminates in CCA is not known. One unexplored aspect is its influence on the host microbiome. In the Syrian hamster, infection with this pathogen reliably leads to CCA. Genomic DNAs of microbiota from colorectal contents and bile of hamsters and *O. viverrini* were examined in this model of fluke-induced CCA. Sequences of regions 7, 8 and 9 of prokaryotic 16S rRNA genes were amplified, pyrosequenced, operational taxonomy units classified, and analysis of community diversity undertaken. Of ~1,000,000 sequences, 536,009 could be assigned to 20 phyla and 273 genera of bacteria or Archaea. Diversity analyses revealed that fluke infection perturbed the gastrointestinal tract microbiome, increasing Lachnospiraceae, Ruminococcaceae and Lactobacillaceae while decreasing Porphyromonadaceae, Erysipelotrichaceae and Eubacteriaceae ($p \leq 0.05$). In addition, >60 prokaryote species were detected in the biliary system, which confirmed bacteriobilia and a remarkable community associated with the parasites. These fluke-associated microorganisms included potential pathogens from the *Enterobacteriaceae* and Listeriaceae and others from external environments including cyanobacteria and Deinococci. Given that opisthorchiasis is distinguished from other helminth infections by a robust inflammatory phenotype, with conspicuously elevated interleukin 6, and that inflammation of the biliary system leads to periductal fibrosis that is a precursor to CCA, the flukes as well as their microbiota might together drive this distinctive immune response.

1490

IMMUNOMICS-BASED IDENTIFICATION OF SCHISTOSOMIASIS VACCINE ANTIGENS: AN INTEGRATED DISCOVERY AND VALIDATION APPROACH

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Schistosomiasis is a neglected tropical disease affecting >230 million people and causes over 200,000 deaths annually. To identify new vaccine antigens and assess their potential protective efficacy and safety, we used an immunomics approach with sera from putatively resistant (PR) and chronically infected (CI; stratified by infection intensity) people in a high transmission area for schistosomiasis in Brazil. We selected mostly tegumental *Schistosoma mansoni* and *S. japonicum* proteins, produced them using an *in vitro* rapid translation system (RTS) and printed them to generate the first protein microarray for a multi-cellular pathogen. Arrays were screened to detect IgG subclass and IgE responses; antigens which showed preferential/unique recognition by IgG1/3 from PR individuals were up-selected, and those that were the target of potentially deleterious IgE responses (in terms of vaccine-induced hypersensitivity) were down-selected. We detected strong correlations between the number of antigens recognized and infection intensity for all antibody subclasses, most notably IgE. Surprisingly, PR individuals produced little IgE but instead made robust IgG1/3 responses to a small number of antigens exposed on the parasite surface, highlighting their potential as vaccine antigens. Cluster analysis was performed to identify antigen clusters based on their antibody recognition profiles. Two clusters contained antigens that were preferentially recognized by IgG1/3 of PR individuals but were not major targets of IgE; these clusters included the previously described vaccine candidates Sm-TSP-2 and Sm29, as well as a panel of new antigens that have not been previously described. We have shown here the use of a high throughput immunomics approach to profile antibody responses from PR and CI individuals that has unearthed a suite of novel potentially protective and safe schistosomiasis vaccine antigens. To complement our human subjects-oriented antigen discovery approach, we are also probing arrays with sera from non-human primates that have been vaccinated with irradiated schistosome cercariae.

1491

ELEVATED ARGINASE 1 AND LOW NITRIC OXIDE SYNTHASE 2 PBMC EXPRESSION: EVIDENCE OF ALTERNATIVE MACROPHAGE ACTIVATION IN CHILDREN WITH MALARIA

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We demonstrated earlier that malaria infection is associated with low serum arginine levels and low expression of nitric oxide synthase2 (NOS2) leading to diminished nitric oxide (NO) production and endothelial dysfunction. We established that NO is protective in malaria. The mechanism of diminished NO production is not well understood, but it is likely to be multi¬factorial. Increased metabolism of arginine by arginases and suppression of NOS2 expression likely play roles. Alternative macrophage activation (M2) is initiated by Th2 cytokines such as IL-4, IL-10, and IL-13. M2 activation is also associated with increased arginase1, decreased NOS2 and NO expression by monocytes-macrophages, and likely less resistance to malaria infection and/or malaria disease. The aim of this study was to investigate the markers for monocytes/macrophage in PBMCs from children *Plasmodium falciparum* infection. Children aged 6mo to 9 yr were recruited from Amana and Mwananyamala hospitals in Dar es Salaam, Tanzania, and categorized (modified WHO criteria) as severe malaria (SM), uncomplicated malaria (UM),or healthy control (HC). We prospectively measured PBMC mRNA for arginases 1 and 2, and NOS2 using quantitative RT-PCR.Results were analyzed using Prism 5 software and Mann-Whitney non-parametric comparison analysis. We enrolled 80 SM, 80 UM, and 48 HC participants. There was marked increase in PBMC arginase 1 mRNA in children with malaria compared to healthy controls (4.5 fold in UM and 9.3 fold in SM; p = 0.02 and 0.008 respectively), while NOS2 mRNA was lower in SM and UM than in HC (p = 0.0001) for each comparison. PBMC arginase 2 mRNA was lower in SM compared to HC, but it was not statistically significant (p = 0.89). In conclusion, malaria infection in children is associated with increased arginase 1, and decreased NOS2 and arginase 2 mRNA expressions. This is characteristic of alternative macrophage activation and may partly explain the low serum arginine and diminished NO production in malaria. Assessment of arginase activity in PBMCs (and purified mononuclear phagocytes) during malaria infection is warranted to fully establish the role of alternatively activated monocytes-macrophages in the hypoarginaemia observed during the malaria infection.

1492

IMBALANCE OF INFLAMMATORY AND ANGIOGENIC FACTORS IN EARLY PREGNANCY ARE ASSOCIATED WITH PRETERM BIRTH IN A PROSPECTIVE COHORT OF MALARIA-EXPOSED TANZANIAN WOMEN

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Malaria in pregnancy is associated with several adverse birth outcomes including preterm birth (PTB). PTB is now the leading cause of perinatal mortality globally, however there are currently no diagnostic tools to predict pregnancies at risk of PTB. Based on the hypothesis that altered placental angiogenesis and inflammation early in pregnancy lead to PTB, we examined if levels of inflammatory and angiogenic mediators, measured early in pregnancy (< 27 weeks gestation), were predictive of PTB in a cohort of women living in a region of high malaria transmission. Plasma samples were collected from a prospective cohort of 432 primigravid women at enrollment (12-27 weeks gestation). A total 63 women subsequently delivered preterm (< 37 weeks gestation). Levels of 18 biomarkers reflective of angiogenic and/or inflammatory pathways (Ang-1, Ang-2, Ang-L3, VEGF, sFLT-1, sTNFR2, PlGF, MIP-1β, MCP-1, Leptin, IL-1β, IL-18 BP, sICAM-1, FAC-D, sEndoglin, CRP, CHI3L1, C5a) were analyzed by ELISA. Plasma levels of PlGF (*P*=0.04), IL-18 BP (*P*=0.002), sICAM-1 (*P*=0.03), sEndoglin (*P*=0.0005), CHI3L1 (*P*=0.002), sTNFR2 (*P*=0.05) were higher at enrollment in women who subsequently experienced PTB compared to women who delivered at term. Based on multiple analytic methods, plasma levels of IL-18BP, CHI3L1 and sEndoglin were elevated at enrollment, in women who went on to deliver preterm. Combinatorial strategies were applied in an attempt to improve predictive accuracy. Combining biomarker data (sICAM-1 and CHI3L1) with clinical and demographic data improved our predictive model of PTB over that possible with clinical data alone (*P*=0.0002). In conclusion, in this cohort of Tanzanian women, levels of angiogenic and inflammatory mediators measured early in pregnancy, were associated with subsequent PTB. These proteins provide insight into the underlying mechanism of PTB and may have clinical utility as early biomarkers of preterm delivery. Given the high rates of PTB in malaria endemic regions, there is a critical need to develop early diagnostic tools to identify pregnancies at risk of PTB.

1493

PLACENTAL MALARIA INDUCES EXCESSIVE VASCULOGENESIS

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Placental malaria (PM) results from sequestration of *Plasmdium falciparum*-infected erythrocytes and the resulting inflammatory responses in the maternal placental blood space. PM induces maternal anemia, preterm birth, low birth weight, or stillbirth, especially in primigravidae. PM may also promote local hypoperfusion or hypoxia, inducing neovasculogenesis in the fetal placental compartment. Excessive vasculogenesis can result in chorangiosis (CHOR), defined as at least 10 vascular channels (VC) in at least 10 terminal villi in 10 low power microscopic fields in three discreet regions of the placenta. CHOR is rare but is enhanced in preeclampsia and diabetes and is associated with neonatal morbidity and mortality. To determine the extent to which PM induces CHOR, placentae were collected from 18 consenting primigravidae at two public hospitals in Kisumu, Kenya. Malaria status and PM chronicity were estimated by microscopic examination of placental blood smears and PCR and by placental histology. Patients were separated into the following groups: uninfected (UN, n=2), active/active chronic (A/AC, n=9) infection, and past/past chronic infection (P/PC, n=7). Thin sections from three fixed placental tissue sections were hematoxylin and eosin-stained, and thirty micrographic images at 200X magnification (to approximate ten low power fields at 100x) were captured from each section. Number of villi and VC therein were counted. Whereas UN and P/PC placentae were equivalent, A/AC placentae had statistically significantly higher median numbers of VC/villus (*P*<0.0001). Moreover, the latter group also had a significantly higher percentage of villi with ten or more VC relative to the other two groups (27.2±1.6% versus 20.5±0.8% (UN) and 20.7±2.0% (P/PC), *P*=0.0355). Further analysis of these samples is underway to evaluate whether these samples meet the clinical definition of CHOR and associations with birth outcomes. In addition, assessment of markers of angiogenesis promises to provide insight into the molecular mechanisms underlying this phenomenon in PM.

1494

RETINAL MICROVASCULAR DYSFUNCTION IN PEDIATRIC CEREBRAL MALARIA IS ASSOCIATED WITH DEATH AND NEUROLOGICAL SEQUELAE

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Malarial retinopathy (MR) appears to reflect brain pathogenesis in pediatric cerebral malaria (CM), since it is related to mortality and highly predictive of brain histopathology. MR is associated with abnormal fluorescein angiography (FA), but mortality associated with these abnormalities is unknown. We aimed to characterize FA abnormalities and their relationship to clinical outcomes. Two ophthalmologists graded admission angiograms in 170 consecutive patients with pediatric CM from 2006 to 2010 (WHO criteria, including retinopathy negative cases). Variation between eyes was assessed using Cohen's kappa statistic. Associations between FA abnormalities, mortality, and presence of neuro-disability on discharge were assessed using left eye data and Fisher's exact test. In our series 118 survived, 25 survived with neuro-disability, and 27 died. All FA signs were consistent between right and left eyes. Frequencies of features were: Capillary non-perfusion (CNP), macular 82%, peripheral 84%; Intravascular filling defect (by vessel type): large 38%, small 14%, occluded 22%; Vessel leak: small macular 56%, small peripheral 49%, mid/large 16%, disc 52%. Severity of macular CNP (p=0.02) and presence of peripheral small vessel leakage (p=0.03) were significantly associated with death and neurological disability; peripheral CNP (p=0.45), and macular small vessel leakage were not (p=0.09). This is the largest analysis of retinal angiography in pediatric CM to date. Retinal CNP is very common. Severity of macular CNP, and the presence of fluorescein leakage from small peripheral retinal vessels are associated with death and neuro-disability. CNP indicates ischemia and matches areas of retinal whitening seen clinically. This result is consistent with a known association between macular whitening and death. FA leakage results from breakdown of the blood-retinal barrier, which is similar to the blood-brain barrier. Our results suggest that central nervous system ischemia and leakage across blood-tissue barriers may be important contributors to the severity of pediatric CM.

1495

FATAL PEDIATRIC CEREBRAL MALARIA IS ASSOCIATED WITH INTRAVASCULAR INFLAMMATION AND COAGULATION THAT IS EXACERBATED BY HIV-1 CO-INFECTION

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Most malaria deaths occur in sub-Saharan African children and are due to various severe malaria syndromes, including cerebral malaria (CM). In Malawi, the overall prevalence of HIV-1 is 10%, with lower seroprevalence in children. The entire population is at risk for malaria. High rates of malaria/HIV co-infection are likely but effects of HIV on CM pathogenesis and outcome are unknown. The Blantyre Malaria Project (BMP) has found 3 patterns of brain pathology in children who met clinical criteria for CM: sequestration alone (CM1), sequestration plus intra- and peri-vascular pathology (CM2) and no sequestration (CM3). In the BMP cohort, the HIV+ rate is 13% overall and 20% in autopsied patients. 60% of autopsies with the CM1 pattern are HIV+ compared to 18% with CM2 and 6% with CM3. To determine whether HIV co-infection affects the pathophysiology of CM, we performed immunohistochemistry on brain tissue from autopsied patients with clinically-defined CM. We examined 10 cases with the CM1 pattern, 10 with CM2 and 10 with CM3 or coma of other cause (COC). Five from each group were HIV+. Brain sections were labeled for HIV-1 p24, ionized calcium binding adapter molecule 1 (Iba1), a marker for microglia and monocytes, and CD61, a platelet marker. No HIV-1 p24 was seen. We observed intravascular Iba1+ monocytes containing hemozoin that completely filled small vessels and adhered to the walls of larger vessels, accompanied by platelet clumps. This was significantly increased in CM1/2 cases compared with CM3/COC cases and was significantly increased in HIV+ CM1/2 cases compared to HIV- CM1/2 cases. Most HIV+ CM1/2 cases had mild immunosuppression by WHO HIV clinical staging and the total lymphocyte counts of HIV+ CM1/2 cases were similar to those of HIV- CM1/2 cases. HIV+ CM1/2 cases were significantly older than HIV- CM1/2 cases. We hypothesize that the intravascular inflammation and coagulation seen in CM autopsies contribute to the pathogenesis of pediatric CM and that dysregulation of these processes in HIV infection contribute to CM mortality.

1496

MALARIA PIGMENT (HEMOZOIN) AND EXTRAVASATED FIBRINOGEN ARE ASSOCIATED WITH RETINAL VESSEL LEAKAGE AND HEMORRHAGES IN MALAWIAN CHILDREN WITH CEREBRAL MALARIA

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Malarial retinopathy (MR) distinguishes cerebral malaria (CM) from non-malarial causes of coma. White-centered retinal hemorrhages are a common clinical feature and vessel leakage due to blood-retina barrier breakdown is found angiographically. We conducted a *post mortem* clinicopathological study to localize features of microvascular pathology affecting neural retina in Malawian children. Histopathological analyses were carried out on 7 cases: 5 cases with clinically defined CM during life showed MR features, and 2 patients with non-malaria comas had no evidence of MR. Retinal microvascular pathology was assessed by presence of: i) extraerythrocytic hemozoin (HZ) in retinal capillaries and venules, on the basis of hematoxylin-eosin staining (H&E); ii) perivascular leakage, with anti-fibrinogen (FGN) and anti-albumin immunohistochemistry (IHC); iii) retinal hemorrhages, with H&E and specific IHC markers (CD45 for inflammatory cells; collagen, smooth muscle actin, CD34 for vessels remnants). The MR cases were classified in two groups: one case had 16% vessels with HZ (Group 1), and four cases showed HZ in a median of 49% (min-max 25-82%) vessels (Group 2). Group 1 showed patchy focal leakage only in the retinal venules with HZ, and no hemorrhages. Each case in Group 2 presented ≥ 5 retinal hemorrhages, characterized by a white-center of FGN which accumulated in the perivascular space together with HZ and inflammatory cells. In the non-MR controls HZ was absent, and one case had retinal hemorrhages secondary to head injury and intracranial hemorrhage (Terson syndrome). HZ was found associated with features of retinal vascular pathology in severe MR cases, concurring with evidence of CM vascular pathology in the brain such as ring hemorrhages. Extravasated FGN from venules with HZ, as well as its presence in the center of retinal hemorrhages, suggest leakage can evolve with disruption

of retinal layers. Further studies on a potential temporal link between the two features can help us to define consequences of blood retinal barrier breakdown in MR.

1497

NON-INVASIVE PULSE OXIMETRY TO PREDICT MORTALITY IN AFRICAN CHILDREN WITH MALARIA

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Between February 2012 and April 2013 we enrolled 1677 children in a prospective observational study of children 2 months to 5 years admitted to Jinja Regional Referral hospital with a history of fever or an axillary temperature >37.5°C and known disease outcomes. The mean age of children enrolled was 1.65 years old, and malaria was the most frequent reason for admission with 76% of children having a diagnosis of malaria based on microscopy and/or positive 3-band RDT (pLDH/HRP2). The mortality rate for children admitted with malaria was 3.1%. We evaluated whether non-invasive pulse oximetry would predict disease outcome in malaria and compared the findings to venous lactate, an established prognostic marker in malaria. We used receiver operator characteristic (ROC) curves to assess the predictive ability of the biomarkers. The area under the curve (AUC) for the oxygen saturation (Sp02) was 0.69 (95% CI, 0.59-0.80; p<0.0001), and a Sp02 less than 92% was 97% sensitive and 37% specific in predicting mortality. In addition to Sp02, the Masimo Pulse CO-oximeter has the capacity to measure the perfusion index (PI), which is the ratio of pulsatile blood flow to non-pulsatile static blood flow in peripheral tissue. The PI is a more sensitive and objective measure of peripheral perfusion than measuring capillary refill. The PI measured on children's finger tips or toes had an AUC of 0.68 (0.57-0.78, p<0.0001), and a PI less than 0.20 was 98% sensitive and 17% specific in predicting mortality. Initiation of appropriate life-saving measures (oxygen administration, treatment for shock) in children with low Sp02 or a low PI resulted in marked patient improvement. Venous lactate ≥5.5mmol/L had an AUC of 0.80 (09% CI, 0.71-0.90; p<0.0001) and a sensitivity and specificity of 81% and 77%. These data suggest that pulse oximetry alongside assessment of venous lactate may be useful in the triage and treatment of children with severe malaria. Additional advantages in pulse oximetry are low operating costs and real-time patient monitoring.

1498

A NOVEL, KINETOPLASTID-SPECIFIC CAMP SIGNALING PATHWAY - A PROMISING DRUG TARGET

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The signaling molecule cAMP plays crucial regulatory roles in almost all eukaryotic cells. In *T.brucei*, the genetic or pharmacological manipulation of the intracellular cAMP concentration results in severe cytokinesis phenotypes with subsequent cell death. Consequently, the cAMP-specific phosphodiesterases have been validated as excellent drug targets. However, the *T. brucei* orthologue of the major downstream target of cAMP, the cAMP-dependent protein kinase (PKA), is not activated by cAMP, nor have homologues of other known mammalian cAMP effectors been identified. We thus used genome-wide RNAi library screening to select cells resistant to Cpd A, a novel and highly specific PDE inhibitor, which kills bloodstream trypanosomes via elevated intracellular cAMP. Four candidate genes (CARP1-4: cyclic AMP response proteins) were identified, whose depletion confers different degrees of resistance to Cpd A. CARP1, a protein unique to kinetoplastid parasites has two predicted cNMP binding domains, and its depletion resulted in up to 200-fold Cpd A resistance. We suggest that this protein is a primary cAMP sensor and CARP2-4 proteins may be components of a novel cAMP signaling pathway. Binding of cAMP to CARP1, physical and genetic interactions among the CARP proteins, and their subcellular localisation are under investigation. We propose the novel kinetoplastid-specific cAMP signaling cascade as promising new drug target for Human African Trypanosomiasis and possibly other kinetoplastid diseases.

1499

CHARACTERIZATION OF THE SMALL PROTEOME OF *TRYPANOSOMA BRUCEI*

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Advances in genomics research are providing new avenues to a more holistic understanding of pathogens. An RNA-Seq transcriptome study from our lab identified 1,114 novel transcripts in *Trypanosoma brucei* of which 993 have at least one potential ORF. The majority fit into the category of short ORFs (sORFs), since the predicted protein is between 25 and 100 amino acids in size. Mining mass spectrometry data sets revealed 42 novel transcripts that encode a sORF matching to at least one unique peptide, suggesting that these proteins are expressed. Thus, the trypanosome proteome appears larger than previously believed. To begin to address the possible function of small proteins in *T. brucei*, all 42 novel transcripts were down-regulated by RNAi and 7 were determined to be essential in procyclic trypanosomes. Each lethal phenotype was rescued by co-expressing an RNAi-resistant construct, further validating the significance of these small proteins. The 7 essential sORFs are only found in trypanosomatids: five are widespread, while two are specific to African trypanosomes. For example, the essential protein encoded by Tb10.NT87 is 64 amino acids long and localizes to the matrix of the mitochondria, as shown by immuno EM, and a karyopherin-like protein has been identified as a potential interacting partner. On the other hand, Tb11.NT29 encodes 62 amino acids with a predicted trans-membrane domain and is localized on the surface of procyclic- and bloodstream-form trypanosomes. In addition, essential small proteins localize to the nucleolus, cytoplasm, and a perinuclear compartment of the cell, highlighting the diverse biological roles they are likely to play. Experiments are in progress to assess the essentiality in bloodstream form trypanosomes and to identify interacting partners.

1500

FUNCTIONAL VALIDATION OF HOST METABOLIC PATHWAYS AS CRITICAL REGULATORS OF *TRYPANOSOMA CRUZI* AMASTIGOTE GROWTH IN HUMAN CARDIOMYOCYTES

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The intracellular amastigote stage of Trypanosoma cruzi is a critical target for vaccine and drug development for the prevention and treatment of human Chagas' disease - the leading cause of infectious cardiomyopathy. Despite the importance of amastigotes in infection and disease, we have a limited understanding of host factors that contribute to the growth and survival of these parasites. In a recent genome-wide RNA interference (RNAi) screen, host metabolic networks centered around energy production, nucleotide metabolism, pteridine biosynthesis, and fatty acid oxidation were identified as key processes that support T. cruzi infection in HeLa cells (Caradonna et al. 2013. Cell Host & Microbe 13

108-117). As a more relevant in vitro infection model, we are exploiting human induced pluripotent stem cell (iPSC)-derived cardiomyocytes for functional validation studies. iPSC- cardiomyocytes are transcriptionally and electrophysiologically similar to adult cardiomyocytes and amenable to high-throughput RNAi screening applications. Using this system, a number of 'hits' that originally surfaced in our RNAi screen have now been validated in cardiomyocytes, including pyruvate dehydrogenase kinase 4 (PDK4). PDK4 regulates the fuel utilization balance in mammalian cells where depletion results in reduced fatty acid oxidation and reduced parasite growth. Coupling host gene knockdown studies with sensitive extracellular flux measurements in live cardiomyocytes has allowed us to confirm the metabolic phenotypes associated with targeted host gene knockdown. We are currently exploiting this experimental system to elucidate the contribution of host lipid metabolism to T. cruzi amastigote growth and survival. The primary objective of this study is to gain mechanistic insight into the relationship between host metabolism and T. cruzi amastigote growth. This knowledge is fundamental to a broader understanding of intracellular parasitism and will open the door to potential alternative interventions.

1501

ROLE OF TLR9 SIGNALING IN EXPERIMENTAL *LEISHMANIA BRAZILIENSIS* INFECTION

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Infection with *Leishmania braziliensis* causes cutaneous or mucocutaneous leismaniasis in humans. TLR9 expression has been found in granulomas of lesions in *L. braziliensis*-infected individuals. *L. braziliensis* inoculation in mice induces very small lesions that are self-healing whereas deficiency in the TLR adaptor molecule, MyD88, render mice susceptible to infection. The TLR receptor involved has not been identified, prompting us to investigate if TLR9 triggering by the parasite contributes to the strong resistance to infection observed in *L. braziliensis*-inoculated mice. The parasites activated wild-type (WT) dendritic cells (DCs) in vitro, but not DCs derived from TLR9-/- mice. TLR9-/- mice inoculated with *L. braziliensis* exhibited a transient susceptibility characterized by increased lesion size and parasite burden compared to WT mice. Surprisingly, elevated levels of IFNγ were measured at the site of infection and in draining lymph node T cells of TLR9-/- mice at the peak of susceptibility, suggesting that unlike observations *in vitro*, the parasite could induce DC activation leading to the development of Th1 cells in absence of TLR9 expression. Taken together these data show that TLR9 signaling is important for the early control of lesion development and parasite burden, but it is dispensable for the differentiation of Th1 cells secreting IFNγ, and that the high levels of this cytokine are not sufficient to control early parasite replication following *L. braziliensis* infection.

1502

DYNAMICS OF APICOMPLEXAN INNER MEMBRANE COMPLEX

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Unlike most cells, which divide by binary fission, protozoa in the phylum apicomplexa divide by a distinctive process in which multiple daughters are constructed within the mother (schizogony, endodyogeny, etc), using a membrane-cytoskeletal scaffolding known as the Inner Membrane Complex (IMC). The IMC is closely associated with the plasma membrane during interphase, but new daughters develop within the cytoplasm, establishing new IMCs. Daughter IMCs elongate rapidly, partitioning subcellular compartments according to a strict schedule. Newly assembled daughters ultimately emerge from the mother, picking up the maternal plasma membrane, and leaving behind vestiges of the maternal cell that were not incorporated into the daughters. While the maternal plasma membrane remains intact throughout this process the maternal IMC disappears -- is it degraded, or recycled to form the daughter IMC? Exploiting a fluorescently tagged integral membrane protein marker for the IMC (GAP40), we have used live cell imaging, photobleaching-recovery (FRAP), and mEos2 photoactivation to monitor the dynamics of IMC biogenesis and turnover during the replication of Toxoplasma gondii tachyzoites. We demonstrate that formation of the IMC involves two distinct steps: de novo assembly during daughter IMC elongation within the mother cell, followed by emergence from the mother cell and further maturation via recycling of the maternal IMC membrane.

1503

PLASMODIUM FALCIPARUM CDC2-RELATED PROTEIN KINASE (CRK) 4 REGULATES DNASEGREGATION AND THE ONSET OF BLOOD-STAGE SCHIZOGONY

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A hallmark of Plasmodium life-cycle progression is a sequence of invasive and replicative stages. Intrahepatic- and intraerythrocytic-proliferation is achieved through schizogony, where a multinucleated cell is formed after which daughter parasites bud off the mother cell. However, the regulatory proteins involved in schizogony are largely unknown. Employing the inducible destabilization domain system in a loss-of-function knockdown screen of schizont-stage kinases, we identified the P. falciparum cdc2-related protein kinase (PfCRK) 4 as essential for proliferation. Depletion of PfCRK4 leads to a complete block in early schizogony at a DNA-content of approx. 6N, which is reversible within eight hours. The block at 6N is similar to what is observed following treatment with the anti-folate drug WR99210, which might be indicative of a general cell cycle checkpoint at 6N. Despite several rounds of DNA replication, analysis by microscopy revealed that PfCRK4-knockdown parasites are unable to segregate their chromosomes. This defect is likely due to an impaired division of the spindle pole body in PfCRK4 depleted parasites. Among apicomplexan parasites, CRK4 is uniquely found in Plasmodium spp., and we provide evidence that PfCRK4 is a key regulator at the onset of schizogony.

1504

TLR7-ELICITED REGULATORY B CELLS USE IL-10 TO SUPPRESS AIRWAY INFLAMMATION THROUGH INDUCTION OF CD4+FOXP3+ T CELLS

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Helminths are potent modulators of the immune system using a diverse range of mechanisms. In recent times, a role for helminth-induced regulatory CD19+CD1dhi B cells (Breg) has been identified as regulators of inflammation in mouse models. Using, microarray technology we have analyzed the gene profiles of *Schistosoma mansoni*-elicited Breg. With respect to innate activation of these cells, toll-like receptor 7 (TLR7)

was a significantly upregulated pathway of interest. The use of TLR7 ligands both *in vitro* and *in vivo* demonstrated the generation of Breg - comparable to helminth-elicited Breg - that produced copious amounts of the immunosuppressive cytokine IL-10. In a mouse model of allergic lung inflammation the use of either TLR7 ligands to induce Breg or the adoptive transfer of *in vitro* generated Breg demonstrated a reduction in airway inflammation and an improvement in lung function. Previously, we have shown how helminth-induced Breg can suppress pulmonary inflammation via CD4+FoxP3+ T cells. Here, we have investigated if TLR7-elicited Breg suppresses airway inflammation via CD4+FoxP3+ T cells and whether this effect is dependent on IL-10. Our work demonstrates how deciphering mechanisms by which helminths modulate the immune system can yield specific targets of therapeutic interest.

1505

THE ROLE OF EPIDERMAL KERATINOCYTES IN THE CUTANEOUS IMMUNE RESPONSE TO *SCHISTOSOME CERCARIAE* AND THEIR EXCRETORY/SECRETORY ANTIGENS

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The epidermis is the site of the initial interaction between schistosome parasites and their mammalian host. During invasion schistosome larvae (cercariae) actively penetrate cutaneous tissue via mechanical damage and the release of excretory/secretory (E/S) products containing proteolytic enzymes and glycans. Invasion promotes angiogenesis and differentiation of 'wound healing' leukocytes in the dermis but it is unclear how these events are orchestrated. Since epidermal keratinocytes are innate sensors of cutaneous wounding, we hypothesised that these cells become activated early during schistosome infection leading to changes in the cutaneous immune responses. C57BL/6 mice were exposed to live *Schistosoma mansoni* cercariae via the pinna and dermal and epidermal cells were isolated from the site of infection at 6h, 24h and 96h post-infection. Epidermal non-haematopoietic (CD45-) cells were then phenotyped *ex vivo* via flow cytometry to identify keratinocyte sub-populations. Relative to un-infected skin, a population of epidermal keratinocytes (CD45-CD326-CD34+) was found to increase following infection. The expansion of this population coincided with expression of markers associated with keratinocyte activation and wound healing in skin explants. Cultures of primary murine epidermal keratinocyte also demonstrated an activated response upon exposure to cercariae E/S material *in vitro*. The functional relevance of changes in keratinocyte sub-populations in the epidermis and their activation state was explored via analysis of parallel changes in dermis-infiltrating antigen presenting cells and tissue inflammation. These results suggest that cutaneous non-haematopoietic cells, particularly keratinocytes, may be important mediators of the early innate immune responses to schistosomiasis *in situ*.

1506

LECTIN AND C2-KINASE SIGNALING REGULATE TROGOCYTOSIS-LIKE INGESTION AND HOST CELL KILLING BY *ENTAMOEBA HISTOLYTICA*

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Entamoeba histolytica is the causative agent of amoebiasis, a diarrheal disease that is a major source of morbidity and mortality in the developing world. Pathogenesis is associated with profound tissue destruction, manifesting as intestinal ulceration or extraintestinal abscesses. Parasite cytotoxic activity is central to tissue destruction, but the mechanism for killing of host cells was unknown. Recently, by employing live confocal fluorescence microscopy, we discovered that amoebae kill by biting off and ingesting distinct pieces of living human cells. The process is reminiscent of trogocytosis (Greek trogo-, nibble) between immune cells. Amoebic trogocytosis initiates within one minute of host cell contact and precedes cell death, as assessed by permeabilization and DNA fragmentation. By using imaging flow cytometry to simultaneously quantify ingestion and killing, we find that pharmacological inhibitors of trogocytosis reduce host cell death in a dose-dependent manner. Trogocytosis is relevant to disease pathogenesis, since we demonstrated using live two-photon microscopy that trogocytosis occurs during invasion of colon explants from fluorescent-membrane mice. We are currently employing dominant negative mutants and recently developed gene knockdown approaches in E. histolytica, in order to define the pathways regulating trogocytosis. Interestingly, a C2 domain-containing protein kinase, EhC2PK, is required for both trogocytosis and conventional phagocytosis in E. histolytica, suggesting that some aspects of conventional phagocytic machinery may be common to trogocytosis. We are using these and other trogocytosis mutants as valuable tools to further dissect tissue invasion and destruction in animal models of infection. Finally, it is notable that the closely related parasite E. dispar is also capable of trogocytosis, and it has been suggested that trogocytosis occurs in Naegleria fowleri. Therefore, not only do these studies change the existing paradigms for cell killing and issue destruction in amoebiasis, they also suggest an ancient origin of trogocytosis as a form of intercellular exchange.

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