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1

ARTEMISININ-BASED COMBINATIONS FOR TREATING UNCOMPLICATED MALARIA IN AFRICAN CHILDREN: THE 4ABC TRIAL

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Artemisinin-based combination therapies (ACTs) are the recommended treatments for uncomplicated *Plasmodium falciparum* malaria. An increasing number of malaria endemic countries in sub-Saharan Africa have adopted ACTs as first line treatments, the most commonly used being arthemeter-lumefantrine (AL) and artesunate-amodiaquine (ASAQ). Such decision was based on a relatively limited information. A clinical trial with the aim of making a 'head-to-head' comparison of four different ACTs was carried out in 10 sites distributed in 7 African countries (Burkina Faso, Gabon, Mozambique, Nigeria, Rwanda, Uganda and Zambia), representing different levels of malaria endemicity. Between July 2007 and December 2008, 4114 children 6-59 months old with clinical malaria (fever and/or history of fever, *P. falciparum* monoinfection with density between 2,000-200,000/µl and Hb ≥ 7.0 g/dl) were recruited and randomized to either AL, ASAQ, dihydroartemisinin-piperaquine (DHAPQ) or chlorproguanil-dapsone plus artesunate (CD-A). They were actively followed up for 28 days and then passively for the next 6 months. AL was administered to 1226 children, ASAQ to 1002, DHAPQ to 1473 and CD-A to 413. Recruitment for the CD-A arm was interrupted in February 2008 following the decision to stop its development. Preliminary results show that day 28 PCR-uncorrected cure rates were 91.1% for DHAPQ, 81.7% for ASAQ, 73.4% for AL and 57.2% for CD-A, with major variations between sites. Day 28 PCR-adjusted cure rates were 98.5% for DHAPQ, 98.0% for ASAQ, 96.8% for AL and 87.4% for CD-A. Using the predefined rule of non-inferiority, a 10% absolute difference in the 28 PCR-adjusted cure rates recalculated to an odds-ratio scale and pooled over the study sites, DHAPQ, AL and ASAQ show equivalence while CD-A appears to have a lower efficacy. Thirteen deaths were recorded during the whole study period, 4 during the first 28 days, one for each study arm. These results show that DHAPQ, AL and ASAQ have similar and excellent efficacy though DHAPQ may exert a stronger post-treatment prophylactic effect.

2

EARLY AND LATE EFFECTS OF TWO ARTEMISININ BASED COMBINATION THERAPIES IN THE TREATMENT OF *FALCIPARUM* MALARIA IN NIGERIAN CHILDREN

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Most antimalarial studies are designed to evaluate peripheral blood smears on a daily basis. This makes it impossible to observe the effects of Artemisinin based therapies in the first 24 hours after therapy. We designed a protocol to evaluate the early effects and the standard measures of efficacies of two ACTs on asexual parasitemia during a drug efficacy study. Children aged 12 months to 132 months were randomized to receive Artemether-Lumefanthrine (AL) or Artesunate-Amodiaquine (AA). Peripheral blood smears were made at 0, 1, 2, 4, 8, 16, and 24 hour, and daily on days 2-7, 14, 21, 28, 35, and 42 for microscopic identification and quantification of asexual *Plasmodium falciparum*. A total of 193 children were randomized to receive either AL (97) or AA (96). A proportion of the children (42% AL, 36.7% AA, p-value 0.377) had a significant rise in peripheral parasitemia that peaked at 1 hour after treatment, followed by rapid decline and clearance. This rise in parasitemia was significant (p= 0.007) and suggests a mobilization of asexual parasites from the deep tissues to the periphery. This finding was unexpected. The other children had the expected pattern of rapid fall in asexual parasitemia until clearance. Fever and parasite clearance times for AL and AA were 29.9 ± 18.4, 28.6 ± 18.4 hours and 28.9 ± 12.7, 24.0 ± 15.4 hours (p= 0.630, 0.067) respectively. There was no record of early treatment failure and cure rates at day 42 was >95% for both drugs. In conclusion, the study showed high efficacy of AL and AA in Nigerian children. Results from this study also suggest a mobilisation effect on parasites from deeper tissues to the peripheral blood in the early hours after drug administration. The biological and pharmacological importance of this observation is yet to be understood.

3

LONGITUDINAL TRIAL OF CHLOROQUINE MONOTHERAPY AND COMBINATION THERAPY FOR UNCOMPLICATED *FALCIPARUM* MALARIA IN CHILDREN IN BLANTYRE, MALAWI

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We previously reported that chloroquine-susceptible malaria returned and predominates in Malawi after the successful removal of chloroquine drug pressure. To investigate strategies for the rational combination of drugs to deter the emergence and spread of resistance, we conducted a longitudinal trial of chloroquine alone and in combination with partner drugs with different pharmacokinetic and pharmacodynamic properties. Children ages six to 59 months who presented to the district health center with clinical, microscopy-confirmed *Plasmodium falciparum* malaria were enrolled in the study and randomized to receive one of the four treatment arms: chloroquine monotherapy, or chloroquine in combination with artesunate, azithromycin or atovaquone-proguanil. Each time a participant developed symptomatic malaria during one year of follow up, they were treated with the same regimen. The primary outcome was incidence of malaria. Other important outcomes included the efficacy of the treatment at the first and subsequent episodes of malaria and the effect of each treatment on hemoglobin at the end of the study period. Six hundred forty children were enrolled. Preliminary results suggest that all treatment arms, including chloroquine monotherapy, were highly efficacious for the treatment of *P. falciparum* malaria and maintained efficacy throughout the trial period. Assessment of the incidence of malaria and of the effect of each treatment regimen on the prevalence of anemia and the re-emergence of chloroquine-resistant genotypes are underway and will be presented. Potential future uses of chloroquine in Africa will be discussed.

4

EFFICACY OF IPTI WITH SULPHADOXINE/PYRIMETHAMINE COMBINED WITH EITHER AMODIAQUINE OR ARTESUNATE ON MALARIA-RELATED MORBIDITY IN AN AREA OF PAPUA NEW GUINEA WITH SIGNIFICANT LEVEL OF NON-*FALCIPARUM* INFECTIONS

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Intermittent Preventive Treatment of malaria in infants (IPTi) is one of the most promising preventative interventions to reduce the burden of malaria in one of the highest risk groups in endemic countries. The concept is to deliver antimalarial treatment to infants at regular intervals, alongside the expanded program of immunization, regardless of the parasitemia. This intervention has been exclusively investigated in Africa where *Plasmodium falciparum* (Pf) is the predominant species. The potential benefits of this intervention in areas with significant levels of non-Pf infections (mainly Pv) is not known. Between June 2006 and June 2010 we conducted a 3 arm randomized controlled trial (part of the IPTi consortium) to investigate the efficacy of IPTi with sulfadoxine/pyrimethamine (SP, single dose) associated to 3 days of either amodiaquine (AQ) or artesunate (ART) compared to placebo in Papua New Guinea (PNG), a country highly endemic for both Pf and Pv. In total, 1125 infants were enrolled and followed-up from 3 to 27 months. As of April 2010, >1800 clinical malaria episodes confirmed by rapid diagnostic antigen testing (RDT) were observed of which *P. vivax* accounting for just over 50%) and 287 serious adverse events (SAE) occurred including 9 deaths (none of them study related). Final results on the efficacy and safety of IPTi for the prevention *P. falciparum* and *P. vivax* clinical malaria, anaemia and hospitalisation will be presented. The results of this study will provide the first evidence for the efficacy of IPTi in settings endemic for both *P. falciparum* and *P. vivax* and have important implication for the role of IPTi as an intervention against malarial outside Africa.

5

EXTENDED PARASITES CLEARANCE TIME AMONG PATIENTS TREATED WITH ARTEMETHER/LUMEFANTRINE OR AMODIAQUINE PLUS ARTESUNATE AT ONE SENTINEL SITE IN TANZANIA

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Drug resistance which is usually preceded by an intermediate stage whereby parasites become tolerant to therapeutic levels of the drugs has presented many challenges to malaria control programmes. Resistance can be detected using standard methods while tolerance can be indirectly revealed by *in-vitro* tests or examining increased parasite clearance time *in-vivo*. Due to high resistance to SP, Tanzania adopted artemether-lumefantrine (ALu) in November 2006 as a first line antimalarial and monitoring of drug resistance is regularly done at sentinel sites to provide data for updating antimalarial drug policy. A study to assess *in-vivo* efficacy of ALu and amodiaquine plus artesunate (AQ+AS) was conducted between April and December 2007 at Mkuzi and Ujiji sentinel sites. Children with uncomplicated *falciparum* malaria (aged 6-59 months) and meeting inclusion criteria were randomized to receive either ALu (n=233) or AQ+AS (n=232); and were followed-up for 28 days. Filter paper blood spots were collected for PCR analysis to distinguish re-infection from recrudescence. At Mkuzi, 82.8% of the cases on ALu and 87.5% in the AQ+AS group had not cleared parasites by day 2; and on day 3, 39.8% in ALu and 25% in AQ+AS still had parasites. At Ujiji, 94% of the cases (in both arms) had cleared parasites by day 2 and only 1 case in AQ+AS group had parasites on day 3. The median parasite clearance time was 3 days at Mkuzi and 1 day at Ujiji. On day 28, PCR corrected adequate clinical and parasitological response (ACPR) in the ALu groups was high but similar at both sites (98.6% at Mkuzi and 100% at Ujiji, p=0.321). In the AQ+AS groups, PCR corrected ACPR on day 28 was significantly lower at Mkuzi than Ujiji (93.4% at Mkuzi *vs* 100% at Ujiji, p=0.033). In conclusion, extended parasite clearance time at Mkuzi in both drug combinations, might indicate early signs of parasite tolerance. However, ALu was efficacious at both study sites while AQ+AS was less efficacious at Mkuzi where malaria transmission is high. Further studies are needed to monitor possible development of parasite tolerance/resistance to ACTs at these and other sites.

6

POPULATION PHARMACOKINETICS OF ANTIMALARIAL DRUGS IN THE TREATMENT OF PREGNANT WOMEN WITH UNCOMPLICATED MALARIA

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Pregnancy has considerable effects on the pharmacokinetic properties of many of the drugs used to treat uncomplicated *falciparum* malaria. Several studies have shown reduced antimalarial drug concentrations in later pregnancy. The reductions are often substantial, and as a result, antimalarial cure rates in pregnancy tend to be lower. Unfortunately, pregnant women are especially vulnerable to malaria and the fetus is adversely affected. No reports have described the pharmacokinetic properties of piperaquine, amodiaquine or desethylamodiaquine in pregnant women with uncomplicated malaria. A pharmacokinetic study were conducted in Thailand (24 pregnant and 24 non-pregnant women) and in Sudan (12 pregnant and 14 non-pregnant women). These studies investigated the pharmacokinetic properties of piperaquine after a standard oral three-day fixed dose regimen of dihydroartemisinin-piperaquine in patients with uncomplicated *falciparum* malaria. Pharmacokinetics of amodiaquine and its principal biologically active metabolite desethylamodiaquine was investigated in the treatment of vivax infections in 28 pregnant women during pregnancy and again after delivery. Dense venous plasma samples were collected and drug measurements conducted according to published methods. Concentration-time profiles were characterized using nonlinear mixed-effects modeling. Different structural models and the impact of different covariates on pharmacokinetic parameters were investigated in full for all three antimalarials. Population pharmacokinetics of piperaquine, amodiaquine and desethylamodiaquine will be described using a population pharmacokinetic modeling approach. These results will be compared with available literature for a full understanding of any potential pregnancy related changes on pharmacokinetics and the impact of these on the pharmacodynamics.

7

POPULATION PHARMACOKINETICS OF ARTESUNATE AND DIHYDROARTEMISININ FOLLOWING A SINGLE ORAL DOSE OF ARTESUNATE DURING THE 2ND AND 3RD TRIMESTER OF PREGNANCY

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The World Health Organization endorses the use of artemisinin-based combination therapy for treatment of uncomplicated *falciparum* malaria in the second and third trimesters of pregnancy. However, the effects of pregnancy on the pharmacokinetics of artemisinin derivatives, such as artesunate (AS), are poorly understood. We analyzed the population pharmacokinetics of oral AS, and its active metabolite dihydroartemisinin (DHA), in pregnant and non-pregnant women with *falciparum* malaria at the Kingasani Maternity Clinic in the DRC. Data were obtained from 26 pregnant women in the late second or in the third trimester of pregnancy and from 25 non-pregnant female controls. All subjects received 200 mg AS. Plasma AS and DHA were measured using a validated LC-MS method with a lower limit of quantification of 1 ng/mL for both AS and DHA. Estimates for pharmacokinetic and variability parameters were obtained through nonlinear mixed effects modeling with NONMEM 7 software. A simultaneous parent-metabolite model was developed consisting of mixed zero-order, lagged first-order absorption of AS, a one-compartment model for AS, and a one-compartment model for DHA. Complete conversion of AS to DHA was assumed. The model displayed satisfactory goodness-of-fit, predictive ability, and stability. Apparent clearance and volume estimates, with 95% bootstrap confidence intervals, were as follows: 195 L (145 - 316 L) for AS V/F, 895 L/h (782 - 1052 L/h) for AS CL/F, 91.4 L (78.1 - 109 L) for DHA V/F, and 64.0 L/h (55.0 - 75.9 L/h) for DHA CL/F. The effect of pregnancy on DHA CL/F was determined to be significant, with a pregnancy-associated increase in DHA CL/F of 42.3% (18.8 - 69.3%). DHA CL/F did not differ substantially between the late second and the third trimesters of pregnancy. The pregnancy-associated increase in DHA clearance detected in this analysis suggests that higher AS doses would need to be used to maintain similar DHA levels in pregnant patients as achieved in non-pregnant controls.

8

EVIDENCE FOR RIBOSOMAL FRAMESHIFTING AND A NOVEL OVERLAPPING GENE IN THE GENOMES OF INSECT-SPECIFIC FLAVIVIRUSES

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Many viruses harbor sequences that induce a portion of ribosomes to shift -1 nucleotide and continue translating in the new reading frame. The -1 frameshift site typically consists of a 'slippery' heptanucleotide fitting the consensus motif N NNW WWH (where NNN represents any 3 identical nucleotides, WWW represents AAA or UUU, H represents A, C or U, and spaces separate zero-frame codons) followed by a 'spacer' region of 5-9 nt then a stable RNA secondary structure (e.g. a hairpin or pseudoknot). Recently, a ribosomal frameshift site was identified in the Japanese encephalitis virus (JEV) serogroup of flaviviruses that gives rise to a protein (designated NS1') whose origin had previously been an unsolved enigma. The identification of programmed frameshifting in the JEV serogroup of flaviviruses prompted us to look at other flaviviruses resulting in the discovery of a novel coding sequence (designated Fairly Interesting Flavivirus ORF; *fifo*) of 253 to 295-codons that overlaps the NS2A-NS2B coding sequence in the -1/+2 reading frame of all insect-specific flaviviruses. Similar coding regions are not present in the NS2A-NS2B regions of any other flaviviruses. Application of blastp to FIFO revealed no similar sequences in the GenBank database. A conserved G GAU UUY slippery heptanucleotide and 3'-adjacent stable RNA secondary structure at the 5' end of the coding region provide a classical motif for -1 ribosomal frameshifting. The stable RNA secondary structure is either a RNA hairpin (*Culex* flavivirus, Quang Binh virus and Nakiwogo virus) or pseudoknot (Cell fusing agent virus, Kamiti River virus and Aedes flavivirus). Additional evidence for the presence of a novel overlapping gene and subsequent expression of a frame-shift product was provided in (i) reporter assays which confirmed the viability of the proposed translation mechanism and (ii) immunofluorescence assays performed with two separate FIFO-specific antibodies which revealed the presence of proteins containing FIFO antigens in CxFV-infected mosquito cells.

9

CULEX FLAVIVIRUS AND WEST NILE VIRUS IN *CULEX QUINQUEFASCIATUS* POPULATIONS IN THE SOUTHEAST UNITED STATES

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Strains of *Culex* flavivirus (CxFV) and related insect-only flaviviruses have been discovered in mosquitoes worldwide. However, little is known of the interactions between the insect-only flaviviruses, other arboviruses and their mosquito hosts, or the potential public health significance of these associations. The specific aims of this study were to 1) describe the geographic distribution, local prevalence, and seasonal distribution of CxFV and West Nile virus (WNV) in *Cx. quinquefasciatus* in the Southeastern U.S., 2) investigate the potential association between CxFV prevalence and WNV disease incidence in the southeastern USA, and 3) describe the phylogenetic relationship of CxFV isolates from the region. Using ArboNET records, 12 locations were selected across Georgia, Mississippi, and Louisiana that have programs routinely collecting *Cx. quinquefasciatus* for arbovirus surveillance and that represent a range of WNV human case incidence levels. Mosquitoes were collected by CDC light traps and gravid traps from the same sites between July and October 2009. Aliquots of homogenized *Cx. quinquefasciatus* pools were screened for flaviviruses by RT-PCR and subjected to virus isolation on C6/36 cells. Georgia experienced the most CxFV activity of all three states. In Georgia, CxFV infection rates (MLE) increased between July and October, and infection rates were highly variable between and within counties as well as seasonally. CxFV infection rates were highest in Fulton County, GA, reaching 200 infected *Cx. quinquefasciatus* per 1000 at some sites. WNV infection rates (MLE) in Georgia were <1 throughout the summer. CxFV infection rates were not significantly different between Georgia and Mississippi in July, however CxFV was not detected in Mississippi after July, and no CxFV was detected in *Cx. quinquefasciatus* in Louisiana in

any month. CxFV isolates from Georgia were 98% identical to CxFV from Japan, Iowa, and Houston in the NS5 gene. Interactions between WNV and CxFV prevalence will be presented.

10

MOLECULAR CHARACTERIZATION OF EPIDEMIC AND NON-EPIDEMIC ST. LOUIS ENCEPHALITIS VIRUS (SLEV) STRAINS ISOLATED IN ARGENTINA

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SLEV (Flavivirus) is an emerging/reemerging arbovirus in South America causing isolated encephalitis human cases or outbreaks in Argentina and Brazil. In the city of Córdoba (Argentina), during a human SLE outbreak, two genotype III SLEV strains were isolated, belonging to the same genotype isolated 27 years ago in Province of Santa Fe (Argentina). Preliminary results show some biological differences among epidemic (Ep) and non-epidemic (NEp) strains. The factors which lead this emergence are not known. The aim of this project was to characterize and compare molecularly both Ep (CbaAr-4005) and NEp (79V-2533) SLEV strains. A complete genome strategy was designed for both Ep and NEp SLEV strains. A bioinformatic analyze was carried out in order to detect cleavage protease site, genetic distance (GD), nucleotide and aminoacidic substitutions and relative homologies index (RHI). Finally a phylogenetic analyze was realized including 26 SLEV strains nearly complete genome sequence available at GenBank. SLEV complete genome consists of 10963 ntds (ORF=3429 aas). Proteins C, PrM, NS2A, NS2B and NS4B have wide regions with RHI greater than >0.80. The most variables proteins (GD) were NS4B (2.7), NS1 (1.7) and M (1.3). A total of 49 conservative and 20 non-conservative aminoacidic substitutions and one deletion were detected in reference with Kern217 SLEV strain (NC_007580.2) sequence. Among Ep and NEp viral strains we detected 17 aminoacidic changes, which 8 of them were non-conservative and located in proteins E, NS1, NS3 and NS5. It is unknown if detected aminoacidic differences would be related to the biological differences previously observed. The development of reverse genetic system will allow us to understand the meaning of such substitutions. The phylogenetic analysis shows two big clades: North and Central America (NCA) and South America (SA). The analyzed strains isolated in Argentina constitute a subgroup inside the NCA group. Likely, ancestors of genotype III could be introduce and originated the SLEV strains circulating in North and Central America.

11

PHYLOGENY OF TICK-BORNE ENCEPHALITIS VIRUSES IN CENTRAL EUROPE

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Tick-borne encephalitis virus (TBEV) is a member of the genus Flavivirus in the family Flaviviridae. It is transmitted in nature by ticks. As far as known, three subtypes are cirvculating in Europe and Asia. Within the particular subtypes, members exhibit little variability in nucleotide and amino acid sequences. By sequencing complete viral genomes we identified a variable region in the NS2a gene which was used for more detailled phylogenetic characterization of TBEV strains in Southern Germany. Ten new TBEV strains were isolated from ticks of four different regions in South-Eastern Germany. All TBEV strains belong to the Western subtype of TBEV. Furthermore, the phylogeny of sequences of E and NS2a genes (as far as available) of other TBE viruses and of Slovak, Czech and Austrian TBE virus strains were used for comparative analysis. One of the new strains, AS33, exhibited unique nucleic acid and amino acid patterns of the E gene with two amino acid exchanges at positions (E51D; T128I) which have not been detected so far in any other known TBEV strain known to occur in Germany or in any other TBEV strain listed in data bases. A TBEV strain (Haselmühl-1) isolated only in 10 km distance to the AS33 focus did not show the described unique amino acid pattern. About 40 km distant to the east, another TBE focus was detected and two TBEV strains were recovered from ticks. A fourth natural TBE focus was detected some 140 km east from the other foci described foci near the city of Passau at the frontier to Czech Republic and to Austria. This South-Eastern part of Germany is assumed to be one of the two most active TBE endemic areas in Germany. However so far, no sequence data on the circulating TBEV strains were available. The generated sequences of TBEV show that the NS2a gene may be better used for comparative phylogenetic analysis of TBEV than the E gene hwich is currently used for most of these analyses. Using the generated sequence data, several genetic clusters of TBEV in Southern Germany can be distinguished. These data imply that TBEV strains were independantly introduced from Slovak Republic and Czech Republic several times. Furthermore, TBE foci seem to be established independantly from each other, even in closely located regions.

12

POTENTIAL MARKERS OF ATTENUATION OF YF VIRUS AFTER INFECTION OF STEM CELL-DERIVED HUMAN HEPATOCYTES WITH WILD-TYPE ASIBI OR LIVE-ATTENUATED YF17D VIRUS

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Yellow fever virus (YFV) is an arthropod-borne virus belonging to the Flaviviridae family, which causes acute febrile illness with symptoms ranging from mild, non specific syndrome to hemorrhagic fever involving all organs, including the liver in which hepatic lesions are accompanied by an intense inflammation. With the objective of developing an in vitro model to evaluate YFV hepatotropism, we compared the infection of hepatocytes derived from human embryonic stem cells (hES-Hep™002) by wild-type YFV or by the attenuated YF-17D vaccine strain. hES-Hep™002 cells were infected by each virus at an M.O.I of 0.01 or 2. Viral replication was quantified at regular intervals in culture supernatants and expression of cytokines and transaminases (ALT/AST - GST) were followed by ELISA and enzymatic assays. Apoptosis and cell infection rates were measured by flow cytometry analysis and a transcriptomic analysis was done using PCR arrays, focusing on cytokines, apoptosis, cellular stress, and drug metabolism pathways. The PCR arrays identified different markers. At low M.O.I. 17D-infected cells were found to express more IL-5 and IFN-γ than Asibi-infected cells and several genes involved in drug metabolism were activated, while activation of the apoptotic pathway was increased in Asibi-infected cells. At high M.O.I. Asibi-infected cells expressed more IFNα, IL-5 and TNF than 17D-infected cells, and genes involved in cellular toxicity were massively activated. At high M.O.I., cytokine expression differences were also highlighted by ELISA: production of IL-6 and IL-8 were up regulated following infection by YF-17D.

Using a hES-Hep™002 cell model we identified differences in the antiviral responses to infection by pathogenic wild-type YFV or the attenuated 17D strain as well as potential markers predictive for YFV attenuation. The relevance of the hES-Hep™002 cell model needs to be established in comparison with primary hepatocytes.

13

PERSISTENCE OF ANTIBODIES ONE YEAR AFTER A SINGLE INJECTION OF LIVE ATTENUATED JAPANESE ENCEPHALITIS CHIMERIC VIRUS VACCINE AT 12-18 MONTHS OF AGE

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Live attenuated Japanese encephalitis chimeric virus vaccine (JE-CV) was developed to replace first mouse-brain derived JE vaccines. An adult trial has shown that 87% of the participants who were seroprotected at month 6 after JE-CV vaccination were still protected at month 60. The objective of this study was to document persistence of JE-CV-induced antibodies in children. In a 5-year follow-up to a phase 3 trial where 1200 JE-naïve Thai and Filipino children aged 12-18 months were randomized 11:1 to receive a single injection of JE-CV (Sanofi Pasteur, Lyon, France) or a control, we are following a cohort of ~600 JE-CV-vaccinated children to assess long term antibody persistence. Plaque reduction neutralization test (PRNT50) antibody titers against homologous JE-CV will be tested in annual samples. Children with titers >/=1:10 are considered seroprotected against JE. Here we present the first data, obtained one year after vaccination. The seroprotection rate in all 1100 children vaccinated with JE-CV in the phase 3 trial was 95% 28 days after vaccination. Of these, 591 children were enrolled in this follow-up trial in August and September 2009. In this subset, the seroprotection rate 28 days after JE-CV vaccination was 100% (95%CI: 99.4-100) and the geometric mean titer (GMT) was 253 (95%CI: 225-284). One year after vaccination (12±1 month), 88.2% (85.3-90.7) of the 591 children were still seroprotected and the GMT was 77.2 (67.7-88.0). No cases of Japanese encephalitis and no vaccine related SAEs occurred during the year after vaccination. In conclusion, all the subjects enrolled in this long-term follow-up presented with seroprotective antibody titers one month after a single injection of JE-CV between the ages of 12 and 18 months; more than 88% of them were still seroprotected one year later. This ongoing follow-up study is documenting the long term persistence of antibodies against JE after a single injection of the new JE-CV vaccine. Data obtained each successive year will enable us to refine the antibody persistence curve.

14

SURVEILLANCE OF ARBOVIRUSES IN FIVE PRIMARY HEALTH CENTERS IN JAKARTA, INDONESIA (2005-2006)

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Although dengue and chikungunya viruses belong to different families, both share the same vector *Aedes aegypti*. While dengue virus (DENV) is known to be hyper-endemic in Jakarta metropolitan, little is known about the endemicity of chikungunya virus (CHIKV). A one-year surveillance was conducted at five primary health centers in Jakarta, representing the West, East, North, South and Central districts from May 2005 to June 2006. A total of 377 febrile participants were enrolled into the study. DENV infections were identified in 57 (15.1%) of cases whereas CHIKV infections in 60 (15.9%). All dengue virus serotypes were identified, most predominantly DENV-2 (35.1%), followed by DENV-3 (24.6%), DENV-4 (12.3%) and DENV-1 (7%). The cross-sectional prevalence of DENV infections prior to the current illness was 89.3% and of CHIKV infections was 10.1%. Unlike DENV cases that were identified all year round (endemic) in all districts, CHIKV cases were only found in December 2005 and February 2006 in South Jakarta and May 2006 in East Jakarta. No DENV and CHIKV co-infections occurred and no fatalities were reported.

15

WORLDWIDE PHYLOGEOGRAPHIC PATTERNS OF DOMESTICATION AND VECTOR COMPETENCE IN *AEDES AEGYPTI*

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Aedes aegypti is a human commensal mosquito that has invaded much of the tropical and subtropical world over the past few centuries. As the principal vector of both dengue fever and yellow fever, this species is enormously important from a public health standpoint. Though *A. aegypti* is often treated as a homogenous species in its role as a disease vector, in reality, the species displays vast morphological and ecological heterogeneity. The two described subspecies of *A. aegypti* (*Aedes aegypti aegypti* and *Aedes aegypti formosus*) differ markedly in their association with human habitats, as well as in their ability to transmit dengue viruses. We have used 12 microsatellite markers, as well as nuclear sequence data, to describe the worldwide population genetics of *A. aegypti* and explore the evolution of human association in this species. Data have been collected from over 30 populations across five continents. Our results suggest that the African sylvan subspecies, *A. a. formosus*, is indeed ancestral to the worldwide domestic form of the species, but that close human association has likely evolved multiple times independently. We have found that most populations of domestic *A. aegypti* across Africa are genetically more similar to *A. a. formosus* than to the worldwide form, *A. a. aegypti*, even when morphologically identified as *A. a. aegypti*. As *A. a. formosus* is known to be significantly less competent for dengue viruses, these results may help elucidate differing patterns of epidemic dengue activity in Africa as compared to tropics in other regions of the world. High-throughput capture sequencing is currently being used to explore patterns of nucleotide sequence differences in candidate genes for dengue competence across worldwide populations of the mosquito. In addition, our microsatellite markers can reliably assign individual mosquitoes back to their population of origin, which could be used in conjunction with information about vector competence for dengue viruses to determine the public health significance of new *A. aegypti* introductions.

16

THE IMPACT OF DENSITY-DEPENDENCE ON NATURAL LARVAL POPULATIONS OF *AEDES AEGYPTI*: A TWO YEAR FIELD STUDY IN TAPACHULA, MEXICO

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Dengue fever is transmitted by *Aedes aegypti*. The larvae of this mosquito develop in containers of water in and around houses. In order to design more efficient approaches for controlling *Ae. aegypti* it is critical to understand the factors that regulate larval density within water-filled containers. Although many studies of intra-specific competition have been conducted using larvae of *Ae. aegypti* in the laboratory, few studies have been done in the natural environment of *Ae. aegypti*, and no published studies have critically examined density dependence in natural containers at normal field densities. Additionally, mathematical models that predict *Ae. aegy*pti populations currently lack empirically-based functions for density-dependence. We performed field experiments in Tapachula, Mexico where dengue is a significant public health concern. Data were acquired during 1 dry season and 2 rainy seasons. Containers with natural food and water which already contained larvae were taken from local houses. Containers were divided in half with a tightly fitted piece of Styrofoam, enabling the natural water to be divided between the two sides in equal volume. Larvae from the container were separated by stage

and divided into a low and high density treatment. Larvae were counted and pupae were removed daily. Once adults emerged, wings were cut and measured to determine body size. Results from the first two seasons showed that density had a significant impact on larval survival, resulting in a 15 percent decrease in survival from the low density treatment to the high density treatment. Adults in the low density treatment were significantly larger than adults in the high density treatment. The last season of data will be acquired from June through August, 2010. These data will be added to the previous data and analyzed using paired t-tests. We will also look at differences between the three seasons. The data collected will then be used to assess and improve the density-dependence function in a detailed *Ae. aegypti* model.

17

IMPACT OF ROAD NETWORKS ON THE DISTRIBUTION OF DENGUE FEVER CASES IN TRINIDAD, WEST INDIES

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This study was undertaken to investigate the impact of road networks on the distribution of dengue fever cases in Trinidad, West Indies. Confirmed cases of DHF for the year 1998 were collected and spatially located using a GIS road map of Trinidad. A new digital geographic layer representing these cases was created and the distances from these cases to the nearest classified road category (5 classifications based on a functional utility system) was examined. The road layer was then decomposed into 5 subsets, each representing 1 of 5 assigned road classifications. The distance from each spatially located DHF case to the nearest road in each of the 5 road subsets was then calculated and placed into 1km bins depending on their distance value. A threshold representing the maximum number of bins was determined by examination of each of the 5 layer's distance histogram. The distances from each spatially located DHF case to the nearest forest land cover was also calculated and divided in 1km bins and then further subdivided into the nearest road class to each DHF case within each bin. Statistical ANOVA and T-tests were performed to determined significance relationships between DHF cases and their distances from the different classifications of road. A positive correlation was found between road networks and DHF cases. More specifically, results showed that dengue cases are more associated with close proximity to minor motorways and greater distances away from forests, especially 3rd and 4th road classifications than with major motorways, 1st and 2nd roads classifications. In conclusion, minor motorways and distance away from forest provide conducive conditions for *Aedes aegypti* dispersal, finding suitable habitats and blood meals required for completion of their gonotrophic cycles. It is recommended that health authorities take these findings into consideration when planning and implementing strategies for the eradication of *Ae. aegypti* in Trinidad.

18

DENGUE VIRUS INTERACTS WITH MOSQUITO SALIVARY PROTEINS AS MEASURED BY INDIRECT IMMUNOLOGICAL METHODS

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Based on our findings that dengue virus infection in mosquitoes produces an altered array of salivary proteins (many of which were discovered to elicit antibody production in people), we anticipated that some such peptides might be directly involved in the transmission of virus to humans. To investigate this, we performed an ELISA-based technique to measure the level of binding of the four dengue virus serotypes with *Ae. aegypti* salivary proteins. The level of virus-saliva binding was determined by using specific monoclonal antibodies against each dengue serotype, as well as by using serum from people with significant levels of antibodies against Aedes spp. saliva (but were otherwise dengue seronegative). When using monoclonal antibodies, we found that dengue 2 presented the highest binding to salivary proteins followed by dengue 4. Dengue 1 and 3 presented similar binding levels to one another, though moderate when compared to dengue 2 and 4, as measured by optical density (OD) when using these monoclonal antibodies. Additionally, when using human serum, dengue 1 and 3 displayed similar binding levels, and to a much greater degree that was seen in dengue 2. These findings suggest that: a) dengue virus is able to bind mosquito salivary proteins, b) there are differences in the binding levels of each serotype to salivary proteins and, c) some immunogenic salivary proteins to humans can also bind dengue virus. This last conclusion may also indicate direct interaction of some salivary proteins with dengue virus and suggest an active role for salivary proteins in dengue transmission.

19

CULEX TARSALIS BLOOD-FEEDING PATTERNS AND HOST PREFERENCE AT A RURAL SITE IN NORTHERN CALIFORNIA

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Culex tarsalis is an important vector of West Nile virus in California, particularly in more rural areas. Based on historical data using serological methods and more recent data using molecular methods, it is a generalist feeder and will take bloodmeals from a variety of avian and mammalian hosts. Like other *Culex* mosquitoes, *Cx. tarsalis* seems to feed more frequently on mammals during the late summer months than during the early summer and spring. To further explore this seasonal change in host selection, bloodfed *Cx. tarsalis* were collected at a rural farmstead in Northern California from June 2008 through June 2009. Engorged mosquitoes were found in all months except November and December. Bloodmeals were identified using either a newly developed Luminex®-based assay or the mitochondrial sequence of cytochrome c oxidase I (COI), and host feeding patterns were assessed. In addition, hosts were censused on three occasions, and host feeding indices were determined for these periods. Host composition in summer months was dominated by four species of colonially nesting Ardeids. In addition to these herons, and while the herons were absent from September to May, the site was populated with various passerine species (that changed throughout the seasons) as well as farm animals including chickens, geese, goats, horses and cattle. When herons were present, *Cx. tarsalis* fed predominantly upon these birds, with heron bloodmeals comprising >80% of the total bloodmeals tested. Of the herons, Black-crowned night-herons, a competent host for WNV, were the preferred host. As the herons left the area, *Cx. tarsalis* feeding shifted to other birds as well as mammals, and in the winter months Yellow-billed magpies and House sparrows, both WNV competent, were the predominant hosts. These data demonstrate that host selection is likely based on a combination of host availability and preference and that WNV-competent hosts are fed upon by *Cx. tarsalis* throughout the year.

20

CONSEQUENCES OF THE EXPANDING GLOBAL DISTRIBUTION OF *AEDES ALBOPICTUS* FOR DENGUE VIRUS TRANSMISSION

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The dramatic global expansion of *Aedes albopictus* in the last three decades has increased public health concern because it is a potential vector of numerous arthropod-borne viruses (arboviruses), including the most prevalent arboviral pathogen of humans, dengue virus (DENV). *Ae. aegypti* is considered the primary DENV vector and has repeatedly been incriminated as a driving force in dengue's worldwide emergence. What

remains unresolved is the extent to which *Ae. albopictus* contributes to DENV transmission and whether an improved understanding of its vector status would enhance dengue surveillance and prevention. To assess the relative public health importance of *Ae. albopictus* for dengue, we carried out two complementary analyses. We reviewed its role in past dengue epidemics and compared its DENV vector competence with that of *Ae. aegypti*. Observations from "natural experiments" indicate that, despite seemingly favorable conditions, places where *Ae. albopictus* predominates over *Ae. aegypti* have never experienced a typical explosive dengue epidemic with severe cases of the disease. Results from a meta-analysis of experimental laboratory studies reveal that although *Ae. albopictus* is overall more susceptible to DENV midgut infection, rates of virus dissemination from the midgut to other tissues are significantly lower in *Ae. albopictus* than in *Ae. aegypti*. For both indices of vector competence, a few generations of mosquito colonization appear to result in a relative increase of *Ae. albopictus* susceptibility, which may have been a confounding factor in the literature. Our results lead to the conclusion that *Ae. albopictus* plays a relatively minor role compared to *Ae. aegypti* in DENV transmission, at least in part due to differences in host preferences and reduced vector competence. Recent examples of rapid arboviral adaptation to alternative mosquito vectors, however, call for cautious extrapolation of our conclusion. Vector status is a dynamic process that in the future could change in epidemiologically important ways.

21

ELIMINATION OF A PRIMARY FILARIASIS VECTOR POPULATION AT AN ENDEMIC FIELD SITE: COMPARING THE RELATIVE VECTOR COMPETENCE OF A NATURAL AND INCOMPATIBLE STRAIN OF *AEDES POLYNESIENSIS*

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Field evaluation of a novel vector control strategy is underway in French Polynesia. In the approach tested, inundative releases of incompatible *Aedes polynesiensis* male mosquitoes infected with *Wolbachia* should result in the sterilization of wild female populations at a field site endemic for filariasis transmission. Prior to the field trial, experiments have compared the release (CP) strain and field population in their fitness, population dynamics and genetic structure, mating competitiveness, and vector competency. Although only males are to be released, the competency experiment is an appropriate precaution to exclude the risk of an increased vectorial competence through introgression crosses or due to unnatural *Wolbachia* infection occurring in the CP strain. Vector competence of the incompatible CP strain was compared with the lab colony from which it was derived. Female mosquitoes of each strain were fed with blood drawn from *Wuchereria bancrofti* positive donors containing low and high densities of microfilariae. To assess the vector competence, *Wuchereria* parasite development was monitored in both strains. Females were screened for microfilariae immediately after the infectious bloodmeal and for infective larvae at the end of the developmental cycle. The strains showed no significant differences in the number of females infected with infective L3 larvae at day-14. However, there was a significant difference in the mean number of infective L3 larvae per female that each strain had permitted to develop. CP females appear less permissive towards filarial worm infection with a reduced degree of infection when compared to natural populations of Ae. polynesiensis. The significance of these observations will be discussed in the framework of field trials designed to assess the efficacy of cytoplasmic incompatibility as a vector control strategy. If successful, this strategy could adequately complement ongoing mass drug administration to augment ongoing efforts to eliminate Lymphatic Filariasis in the South Pacific.

22

A CAUSAL FRAMEWORK FOR EVALUATING PRE-EXISTING INTERVENTIONS: AN EXAMPLE MOTIVATED BY EFFORTS IN THE WATER, SANITATION AND HYGIENE SECTOR IN RURAL INDIA

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Although the gold standard for causal inference from community-level interventions is the community-randomized trial, many interventions cannot be randomized, and the conditions of trials often make their external validity and sustainability difficult to assess. Studies of non-randomized, pre-existing interventions can estimate the average treatment effect among those most likely to receive an intervention from providers who will actually deliver it (a policy-relevant quantity). Pre-existing interventions can also provide useful information about intervention sustainability. However, evaluating such efforts raises challenges for the unbiased estimation of treatment effects. Drawing on a causal inference framework, we developed a matched cohort design to study non-randomized, pre-existing interventions. We illustrate the strengths and limitations of the method with a sanitation mobilization, water supply and hygiene intervention in rural Tamil Nadu, India. In a propensity score matched sample of 25 villages, we enrolled 1,285 children < 5 years, up to 4 years after the end of intervention activities. Over 12 months, we measured sanitation and hygiene practices, water quality, and child health (diarrhea and anthropometry). The matched cohort design resulted in a control group that was extremely similar to intervention villages across numerous covariates. Access to improved water sources was universal in both groups, but intervention households were far more likely than controls to construct toilets (48% v. 15%, p<0.0001). Adults practice daily open defecation in 39% of all households with a private toilet. Diarrhea among children < 5 was rare in all villages: 1.8% combined prevalence over 14,259 child-weeks (adj. longitudinal prevalence difference = -0.002, p=0.69). The stunting prevalence in the cohort was 53%, and there were no detectable anthropometric gains due to the intervention (Z-score differences < 0.07). In concluaion, matched cohort designs can be a useful tool to study non-randomized, pre-existing community interventions that arise in actual development efforts. In some environments, diarrhea prevalence can be very low despite ubiquitous open defecation; even with these surprisingly low diarrhea rates, growth is poor in this cohort.

23

EFFECT OF A LARGE-SCALE SANITATION, HYGIENE EDUCATION AND WATER SUPPLY INTERVENTION IN RURAL BANGLADESH

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Sanitation Hygiene Education and Water supply in Bangladesh (SHEWA-B) is a large program targeted at 19 million rural people. Between 2007 and 2009, the intervention aimed to change 11 key behaviors related to hygiene, sanitation and water supply. Program activities included household visits, tea stall sessions and courtyard meetings, as well as other social mobilization activities. We assessed indicators at baseline and after 2 years, using 5-hour structured observations of hand washing behavior and spot-checks of water sources, latrines, and waste disposal in 500 randomly selected intervention and 500 control households. To assess the health

impact we established sentinel surveillance in 500 intervention and 500 control households. The proportion of people washing both hands with soap or ash after cleaning a child's anus in intervention areas improved from 22% to 36%, significantly better than in control areas (P=0.048). Households in intervention areas without access to latrine facilities, who practiced open defecation, reduced slightly from 10% to 7% (P=0.017). In the intervention areas the proportion of households that reported hearing hygiene messages increased from 72% to 77%, significantly better than in the control areas (P<0.001). There were no significant improvements found in the practice of child feces disposal, improved latrine use, cleanliness of latrine, storing of drinking water in a covered container and waste disposal. Overall, on monthly visits during the first 21 months, 11% of children under 5 years of age in intervention areas were reported to have diarrhea in the preceding 2 days compared to 10% of children in control areas (P=0.67). In concluaion, the large ambitious SHEWA-B intervention improved only a few of its targeted behaviors, and these changes were sufficiently modest that they did not lead to a measurable reduction in childhood diarrhoea.

24

IMPACT OF BASIC CARE PACKAGE DISTRIBUTION ON THE HEALTH OF PEOPLE LIVING WITH HIV/AIDS--ETHIOPIA, 2009

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Opportunistic infections (OIs) cause substantial morbidity and mortality among people living with HIV/AIDS (PLWHA) in Ethiopia. To reduce the risk of OIs, a national program in 2009 provided free basic care packages (BCP) - which included water chlorination products, safe water storage containers, soap, condoms, and albendazole - to PLWHA. To evaluate the impact of BCPs, we enrolled PLWHA from antiretroviral treatment (ART) programs at two hospitals and designated enrollees from one hospital as the intervention group and from the other as the comparison group. We conducted a baseline survey and chart review, then provided BCPs to the intervention group followed by biweekly home visits for 14 weeks to both groups to ask about recent onset of diarrhea, respiratory infection, and febrile illness. We enrolled 405 PLWHA from the intervention group and 344 from the comparison group. At baseline, both groups had similar median CD4 cell counts (279 vs. 265 cells/μL, respectively, p>0.05); 80% and 75%, respectively, had been on ART for >1 year (p>0.05). Over 14 weeks of follow up, we made 2,721 home visits in the intervention group and 2,258 in the comparison group. Intervention group members were less likely than comparison group members to report any illness (13.3% vs. 26.9%, p<0.05) or febrile illness (5.9% vs. 8.9%, p<0.05) in the preceding 48 hours, or to have visited a health facility in the preceding two weeks for any illness (8.5% vs. 14.9%, p<0.05), for diarrhea (0.7% vs. 1.5%, p<0.05), or for respiratory infection (1.0% vs. 2.1%, p<0.05%). The all-cause hospitalization rate (2.2 vs. 6.9 per 1000 home visits, p<0.05) and all-cause mortality rate (0.7% vs. 1.5%, p>0.05) were also lower among intervention than comparison group members. Over the study period, BCP recipients reported fewer illnesses and health facility visits, and had fewer hospitalizations and deaths than PLWHA not receiving BCPs. These data suggest that BCPs can be an effective approach to reducing illness risk in PLWHA. Further research is needed to assess the sustainability of this intervention.

25

LONG-TERM IMPACT OF INTEGRATION OF HOUSEHOLD WATER TREATMENT AND HYGIENE PROMOTION WITH ANTENATAL SERVICES ON MATERNAL HOUSEHOLD HYGIENE PRACTICES IN MALAWI

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The risk of diarrhea, a leading cause of childhood mortality, can be reduced by household water treatment and handwashing with soap. A clinic-based program to integrate distribution of hygiene kits - which contained safe water storage containers, water treatment solution (*WaterGuard*), soap, and hygiene education - with antenatal services was implemented in Malawi in 2007. To evaluate program impact on water treatment and handwashing technique, we interviewed mothers (program participants) 9 months after implementation; to assess diffusion of the intervention into the community, we surveyed relatives/friends identified by program participants. The evaluation demonstrated significantly increased *WaterGuard* use and improved handwashing technique among program participants and their relatives/friends. To assess program sustainability, we conducted an evaluation 3 years after program implementation was initiated. We enrolled 389 participants and 386 relatives/friends at baseline; we surveyed 232 program participants and 168 relatives/friends at 3-year follow-up to assess current water treatment practices, test drinking water for residual chlorine, and observe handwashing technique. We compared follow-up results to baseline data. Program participants were more likely to know correct water treatment procedures (68% vs. 29%, p<0.0001), treat drinking water with *WaterGuard* (25% vs. 2%, p<0.0001), purchase and use *WaterGuard* (23% vs. 2%, p<0.0001), and demonstrate correct handwashing technique (37% vs. 22%, p<0.001) at the 3-year follow-up survey than baseline. Relatives/friends were also more likely to know correct water treatment procedures (50% vs. 27%, p<0.0001), treat drinking water with *WaterGuard* (16% vs. 2%, p<0.0001), purchase and use *WaterGuard* (15% vs. 2%, p<0.0001), and demonstrate correct handwashing technique (30% vs. 18%, p<0.005), at 3-year follow-up than baseline. This antenatal-clinic-based program appeared to be effective at promoting sustained water treatment and proper handwashing technique among program participants and selected relatives/friends.

26

THE IMPORTANCE OF HANDWASHING BEFORE PREPARING FOOD: OBSERVED HANDWASHING AND SUBSEQUENT DIARRHEA IN RURAL BANGLADESH

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Efforts to improve hand hygiene in communities with high levels of child mortality have focused on encouraging community residents to wash their hands with soap especially after defecation. We analyzed data from the control group enrolled in a large prospective project evaluation to assess the relationship between observed handwashing behavior and subsequent diarrhea. Field workers conducted five hour structured observation and a cross sectional survey in 347 households across 50 villages in rural Bangladesh. Each month for the subsequent two years a trained community resident visited each of the enrolled households and collected information on the occurrence of diarrhea in the preceding 48 hours among all household residents under the age of five years. Field workers observed at least one opportunity to wash hands before preparing food in 281 (81%) and at least one opportunity to wash hands after defecation

in 102 (29%) of the households during structured observation. Compared to children living in households where caregivers prepared food without washing their hands, children living in households where caregivers washed at least one hand with water only (odds ratio [OR]= 0.78; 95% confidence interval [CI] = 0.57, 1.05), washed both hands with water only (OR= 0.67; 95% CI = 0.51, 0.89), or washed at least one hand with soap (OR= 0.30; 95% CI = 0.19, 0.47) before preparing food had less diarrhea. In households where residents washed at least one hand with soap after defecation (OR= 0.45; 95% CI = 0.26, 0.77) children subsequently experienced less diarrhea, but there was no association between handwashing with or without soap before feeding a child, before eating, or after cleaning a child's anus who defecated and subsequent child diarrhea. In conclusion, these observations suggest that before preparing food is a particularly important time to promote handwashing to prevent childhood diarrhea, and that hand rinsing without soap can significantly reduce childhood diarrhea.

27

EFFECT OF DAILY ACTIVITIES ON HAND FECAL CONTAMINATION AMONG TANZANIAN MOTHERS: IMPLICATIONS FOR MEASURING HANDWASHING BEHAVIOR

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The lack of reliable handwashing behavior indicators hinders rigorous evaluation of interventions promoting hand hygiene. Fecal indicator bacteria (FIB) levels on hands are used to measure hand cleanliness; however, limited evidence exists regarding how daily activities affect levels of FIB, and whether their occurrence is correlated with the presence of pathogens. A household observational study, combined with hand rinse sampling, was conducted to assess changes in FIB concentrations on hands attributable to typical daily activities among 119 mothers with young children in Dar es Salaam. Twenty-two mothers were observed performing daily activities for an 8-hour period, during which hand rinse samples were obtained every 2 hours. Another 97 mothers were asked to carry out a specific household activity, with hand rinse samples obtained before and after the activity. A "sitting" group was also enrolled as a control. All samples were analyzed for enterococci and *E. coli*, and select samples were analyzed for genetic markers of *Bacteroidales,* enterovirus, and pathogenic *E. coli*. Using the toilet, cleaning up a child's feces, sweeping, washing dishes, food preparation, and bathing were all found to increase FIB on hands, with geometric mean increases ranging from 50 to 6310 colony forming units (CFU) per two hands. Among samples obtained during a mother's daily routine, food preparation, exiting the household premises, and increased time since last handwashing with soap were positively associated with FIB levels. Bathing was negatively associated with FIB. *Bacteroidales,* enterovirus, and pathogenic *E. coli* were all detected on mothers' hands. Given that FIB on hands are influenced by multiple activities commonly performed by mothers throughout the day, single measures of hand FIB should thus be considered highly variable indicators of hand hygiene behavior. This work corroborates hands as vectors of disease; it also highlights the difficulty of maintaining good personal hygiene in an environment characterized by non-networked sanitation and water supply services.

28

LASTING CHANGES IN HAND HYGIENE BEHAVIOR FOLLOWING INTERVENTION AT THE TIME OF ACUTE ILLNESS, KISHOREGONJ, BANGLADESH, 2009-2010

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In 2009 in Bangladesh, we assessed the impact of handwashing promotion on secondary transmission of respiratory illness in 174 households. The intervention emphasized keeping soap at a handwashing place and washing hands after contact with respiratory secretions and feces. To assess whether intervening at a teachable moment, such as acute illness, results in lasting behavior change, we performed a follow-up study to assess handwashing behavior 4-7 months after enrollment. We observed soap at the handwashing place in intervention and control households. During 90-minute structured observations, we observed handwashing at critical times, such as after contact with respiratory secretions, after toileting, and before cooking. We then prompted one respondent from each household to show how s/he coughs or sneezes (respiratory secretion prompt), and prompted caregivers of children < 5 years old to show how they clean the baby's bottom after defecation (fecal contact prompt). We recorded hygiene behaviors reported or demonstrated by respondents after the prompts. Among 170 enrolled households (89 intervention and 81 control), soap was observed at the handwashing place in 34% of intervention and 19% of control households (OR=2.2, 95% CI=1.1-5.6). During structured observation, soap was used at 6% of all critical times for handwashing in intervention households and 2% in control households (OR=2.4, 95% CI=1.2-4.9). After the respiratory secretion prompt, 61% of intervention and 9% of control respondents said they would wash hands with soap (OR=16.3, 95% CI=6.7-39.4); only 2 intervention and 1 control respondents were observed to wash hands with soap. After the fecal contact prompt, 33% of intervention and 31% of control respondents said they would wash hands with soap (p=.75). Only 3 intervention and 3 control respondents, who actually touched the child's bottom after being prompted, were observed to wash hands with soap. In conclusion, our intervention resulted in lasting increase in maintenance of soap at handwashing places. While awareness of the need for handwashing after respiratory secretion contact was higher in the intervention group, there was no difference in observed handwashing with soap after either respiratory secretion or fecal contact. An intervention to prevent transmission from an acutely ill patient to household members may not result in long-lasting handwashing behavior change.

29

UNDERSTANDING PREGNANT WOMEN'S UPTAKE OF MALARIA PREVENTIVE INTERVENTIONS: A SYSTEMATIC REVIEW OF QUALITATIVE RESEARCH

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Malaria infection in pregnancy is of particular concern to public health workers in endemic areas because of the serious consequences for mothers and infants. Effective preventive interventions include insecticide treated nets and intermittent presumptive treatment. International donors heavily promote these important interventions to meet progressive coverage targets; but the real challenges lies in understanding what is important to pregnant women, their response to preventive interventions, and the various influences on their preventive health behaviour. Synthesis

of qualitative research is of emerging importance in global health; it can provide evidence of what is important to communities and can help understand what works in terms of implementing prevention programmes. Systematic reviews help draw out useful lessons for policy and programme decisions makers by bringing together a body of evidence in an accessible format. However, there are few examples of qualitative synthesis applied to global health questions; the methods for synthesising qualitative research are still in development, and important methodological questions remain. This research, funded by the MRC, will delineate to policy makers and programme managers what is currently known about social, cultural and behavioural aspects of pregnant women's uptake of malaria preventive interventions, what gaps in knowledge remain, and the agenda for future qualitative research in this area. We will also contribute to the methodological development of the thematic approach to synthesising qualitative research. The review findings have the potential to help various stakeholders in malaria control to better understand implementation in terms of barriers and facilitators to uptake of preventive strategies by pregnant women.We will report our progress in conducting a systematic review of factors influencing pregnant women's uptake of malaria preventive interventions; we will present the methodology and any preliminary findings.

30

CHILDHOOD ROTAVIRUS MORTALITY IN INDIA

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India has over 2 million child deaths annually and over 300,000 are due to diarrheal diseases. Rotavirus is a major cause of childhood diarrhea, however, direct estimates of mortality from rotavirus in India are not possible. Our objective is to combine mortality data from a nationally-representative study of deaths in India with the regional, age specific, microbiologic data on etiology of severe diarrhea in children to estimate the number of rotavirus attributable diarrheal deaths in children under 5. This study uses data from the verbal autopsy based Million Death Study (MDS). The MDS surveyed 6.3 million people in 1.1 million nationally representative Indian households for vital status between 2001 and 2003. Diarrheal deaths were those with final ICD-10 codes A00 to A09. The fraction of MDS deaths caused by diarrheal diseases was applied to the gender and age specific number of childhood deaths by region within India. Regional, age-specific proportions of hospitalized children with diarrhea that tested positive for rotavirus by enzyme immunoassay from the Indian Rotavirus Strain Surveillance Network were applied to estimate the number of dirrheal deaths attributable to rotavirus. We estimate that nearly 320 000 children died from diarrhea in India in 2005, of which about 65 000 were due to rotavirus. Approximately 50% of the rotavirus deaths occur in the first year of life and 90% in the first two years of life. The majority of rotavirus deaths are in the Central and East regions. At ages 1-59 months, diarrhea and rotavirus death rates were about 40% and 25% higher respectively in girls than in boys. In conclusion, rotavirus gastroenteritis is a major killer of children in India, particularly those under two years of age and girls. New rotavirus vaccines have been shown to be effective at reducing severe rotavirus disease. If the rotavirus vaccine was introduced into India, then several tens of thousands of annual diarrheal deaths could be prevented and the gender inequalities in childhood mortality would be reduced.

31

PSYCHOGRAPHIC FACTORS RELATED TO FEMALE GENITAL CUTTING IN GUINEA

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Ninety-six percent of Guinean women have undergone female genital mutilation/cutting (FGM/C) (DHS, 2005). In 2000 a law was passed forbidding the practice and specifying that those conducting FGM/C could be punished through forced labor or life imprisonment if the practice led to the girl's death within 40 days. Population Services International/Guinea is conducting a program to reduce the practice of FGM/C, and conducted a baseline study to analyze the factors related to intention to practice FGM/C. A mixed qualitative and quantitative approach was used to collect data on FGM/C in Guinea. In 2008, 16 focus groups were conducted with women and men to collect information that was used to generate scale items for a quantitative survey. The household survey was conducted in 2009 and 4,143 caregivers (women and men aged 18-55) of children aged 4-12 were interviewed nationwide. A descriptive analysis was run on the behaviors of interest and a logistic regression was conducted to identify determinants of the intention not to practice FGM/C. The majority of men and women, 55% and 67%, respectively, intended to practice FGM/C on their child. Roughly half of women and men believed that FGM/C was not a good practice. Those who received the social support of their community environment to stop FGM/C were 2.1 times more likely to not intend to practice FGM/C (P<.001) and those who felt that not practicing FGM/C promotes harmony, equity, non-discrimination, health and wealth in the community were likewise 2.1 times more likely to be non-intenders (P<.001). Social norms and beliefs against FGC/M and locus of control for stopping FGC/M were also associated with non-intention. Intention to practice FGM/C was highest in the "Basse Guinea" district (p<.01). In conclusion, despite the law forbidding the practice, FGM/C intent remains prevalent among caregivers. The determinants identified in this study will be used to develop communications campaigns supporting the reduction of FGM/C among the general population and specifically the residents of Basse Guinea.

32

IMPACT OF A MEDICAL MISSION IN THE HEALTH OF CHILDREN FROM FOUR COMMUNITIES OF THE PERUVIAN AMAZON

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Humanitarian assistance (HA) in the form of short-term medical missions (MM) is an important contribution to the immediate needs of populations living in adverse conditions, although there is debate about whether such activities have long-term, population-wide health effects. The US Naval Medical Research Center Detachment (Naval Medical Research Center Detachment) is evaluating the health impact of an annual MM in four riverine communities in the Peruvian rainforest. Two months before and 10 months after the first MM we studied two random samples of children <5y.o. selected after a population census. Caretakers answered a brief survey about the child's health status and trained personnel collected a stool sample and a drop of peripheral blood in children 1-5 years old. Hemoglobin (Hb) was assessed using a digital measurement device, Hemocue®. The reported prevalence of complete immunizations, illness in the last two weeks (fever, cough and diarrhea) and anemia (Hb<11) were compared before and after MM. The study had 80% statistical power to detect differences >10% in any of these markers.The MM provided eight days of medical attention, 3,602 consultations (907 pediatric) and

5,905 prescriptions, including iron supplements and antiparasitic drugs. We studied 453 and 419 children before and after the MM, respectively. Caretakers reported a 6% increase in the frequency of complete immunization coverage after the MM and 6% to 7% increases in the reported prevalence fever, cough and diarrhea in the two weeks before the survey, although none of these differences were statistically significant. However, we observed a significant reduction in the prevalence of anemia (38% vs. 27%, p=0.003) in children 1-5 years old (n=264 and 302, respectively). This reduction was observed separately in all communities but significantly in only one: Saramiriza (27% vs. 25%, p=0.849), Puerto América (33% vs. 19%, p=0.322), Santa Cruz (37% vs. 32%, p=0.642) and Lagunas (43% vs. 27%, p=0.002). Parasite prevalence results will be available at a later date. In conclusion, despite offering extensive medical services this HA activity did not appear to reduce the frequency of childhood infections. However, we observed an 11% reduction in the prevalence of anemia, a trend partially present across all sites. Although other explanations cannot be ruled out, this MM may have increased Hb levels and the possibility of long-term effects of HA deserves further exploration.

33

TREATMENT SEEKING BEHAVIOR OF CAREGIVERS WHO HAVE CHILDREN WITH DIARRHEA IN BOLIVIA

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Diarrhea is a major cause of morbidity and mortality worldwide in children under five years of age. In Bolivia, the child mortality rate is 65 deaths per 1,000 children and diarrhea causes 37% of these deaths. The decisions made by caregivers when their child has diarrhea profoundly impacts their child's prognosis. The goal of this study was to determine the relationship between socio-economic status, illness severity, and rotavirus vaccination status with treatment seeking behavior of caregivers who have children with diarrhea. In order to assess these associations, the study conducted caregiver surveys (n= 622) at hospitals, clinics, and emergency rooms in four of the largest cities in Bolivia to collect demographic information, clinical symptoms, and caregiver treatment seeking behaviors. This study found that socioeconomic status, illness severity, and rotavirus vaccination were associated with treatment seeking outcomes. Family income was not associated with treatment seeking behavior, but, interestingly, perceptions of socio-economic status did influence treatment seeking behavior. Self-reported difficulty to pay for treatment was significantly associated with both place of first treatment and type of appointment at time of interview. Hydration status (illness severity indicator) was significantly correlated with treatment seeking behavior. This analysis contributes to the limited literature on treatment seeking behavior for child diarrhea. The results from the analysis of treatment seeking behavior can be used to allocate limited resources by increasing access to care in order to reduce the social and financial costs associated with childhood diarrhea in Bolivia.

34

THE IMPACT OF HOUSEHOLD RELOCATION ON CHILDHOOD IMMUNIZATION AND PHYSICIAN VISITS IN DHAKA, BANGLADESH

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Residential relocation may influence a household's ability to obtain health care. We undertook secondary analysis of a cross-sectional population-based study in an urban area of Dhaka, Bangladesh to examine the relationship of residential mobility with full immunization and healthcare-seeking practices for acute respiratory infection (ARI) among children <5 years of age. We categorized 10742 randomly selected <5-years old children based on the length of time at their current address: < 1 year/ recently relocated, 4515 (42%); > 2 years/residentially stable, 5527 (51%). The remaining 7% of children fell between the two periods. Field workers interviewed the children's primary caregivers using questionnaires soliciting socio-demographic and health care information. Primary study outcomes were full immunization defined according to the WHO definition, among children 9-59 months of age and physician visits. ARI was defined as the most recent episode where a child experienced symptoms of cough or difficulty breathing with any additional danger sign. Compared to residentially stable children, recently relocated children were younger (29 vs. 30 months), had smaller families (4.6 vs. 5.4), less educated parents, an average monthly household income < U.S $73 (24% vs. 18%) and had less knowledge on the location of local children's hospitals (42% vs. 58%). In addition, one quarter of the children from recently relocated households were from the poorest wealth quintile (24% vs. 17%) and the household head was 1.5 times more likely to earn income through daily wages. Controlling for enabling and socio-demographic confounders, recently relocated children were 16% less likely to be fully immunized [OR: 0.84, CI: 0.73-0.97], and 29% [OR: 0.71, CI: 0.54-0.94] less likely to seek care from physicians when suffering from ARI. In conclusion, urban Bangladeshi children from recently relocated households differed from children in residentially stable households on their uptake of full immunization and health care-seeking practices. Connecting relocated households to the existing health care system for both preventive and curative care may be a low-cost intervention to improve child health.

35

INTEGRATING POPULATION, HEALTH AND ENVIRONMENT: CONNECTING REPRODUCTIVE HEALTH CONCERNS WITH HEALTH INTERVENTIONS IN THE CONTEXT OF CONSERVATION

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Population-health-environment (PHE) is a development approach that recognizes the links between human health, population pressure, and the environment. It is based upon trans-disciplinary collaboration between conservationists and the public health community. We conducted a community-based, general "ecohealth" assessment in the buffer zone of Gorongosa National Park in Mozambique in order to develop interventions that support the park's mission of linking conservation and sustainable human development. As part of the larger ecohealth assessment in the park's buffer zone, we investigated the communities' view of reproductive health and family planning in order to engage the community in developing PHE programs. Trained local moderators conducted twelve focus groups in six communities, segmented by gender, on questions of health and the environment. Focus groups were recorded and then transcribed and translated into English by the trained local translators and moderators. Qualitative analysis software was used to code and organize data. Communities perceived issues of reproductive health to be of importance to their overall health, including the lack of obstetric care and family planning. Lack of information about, and access to, family planning were contributors to disuse of contraception, as was the social desirability of many children and the fear of one's husband. Participants did not explicitly link population growth to local environmental problems or resource scarcity. Community members expressed a desire for family planning and obstetrical services. Providing such services could contribute to improved reproductive health as well as potentially address the impact of local population pressure on the park.

36

INHERITANCE PATTERNS AND RECOMBINATION FREQUENCY IN THE 3D7 X HB3 *PLASMODIUM FALCIPARUM* EXPERIMENTAL CROSS : A NEW GENETIC MAP

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Three experimental genetic crosses have been performed using the human malaria parasite *Plasmodium falciparum*. Linkage analyses of progeny clones of these crosses has allowed the identification of parasite loci defining phenotypes such as the resistance to antimalarial drugs. Genetic maps produced using markers such as microsatellites and SNPs are available for two of the crosses, but a detailed map has not been available for the first genetic cross between parasites 3D7 and HB3. The frequency of recombination has been investigated in only one cross so far, revealing a map unit size of 17kb/cM. Twenty progeny clones from the 3D7 x HB3 cross have been genotyped using a custom-built Affymetrix molecular inversion probe (MIP) 10K malaria panel array to generate a detailed genetic map. The frequency of recombination in this cross differs from that published for the Dd2 x HB3 cross. Crossing over events in the 3D7 x HB3 cross appear to be more frequent, and non-reciprocal gene conversion events less frequent than in the Dd2 x HB3 cross. The new linkage map and parameters of recombination will be presented and comparisons made with the previous crosses to present a broader picture of the extent of recombination within this species.

37

DEVELOPMENT OF A MALARIA VACCINE CONSISTING OF GENETICALLY ATTENUATED PARASITES

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Generating sterile and long lasting protective immunity against malaria parasites with subunit vaccines has been fraught with difficulties. This has renewed an interest in 'whole organism vaccines' consisting of attenuated parasites. Immunization with sporozoites, attenuated by irradiation that invade and arrest during their development inside hepatocytes have been shown to induce strong protective immunity in rodent models of malaria and also, importantly, in humans. Through targeted deletion of genes, or combination of genes, in rodent parasites it has been shown that sporozoites can become similarly as irradiated sporozoites attenuated during liver stage development. Moreover, immunization with these genetically attenuated sporozoites (GAS) results in protective immune responses similar to radiation attenuated sporozoites. This far, two GAS vaccine candidates, Δ*p36&p36p* and Δ*fabb/f*, which can induce full protection against malaria infections in the rodent malaria model, *Plasmodium berghei* are studied in extent. Unexpectedly, we found that not all of these mutants arrested completely, resulting in blood-stage infections subsequent to immunization (i.e. 'breakthrough parasites'). By analyzing liver stage development *in vitro* and *in vivo* with these mutants now generated in fluorescent and bioluminescent backgrounds we demonstrated that these breakthrough parasites originated from small numbers of parasites that developed inside liver cells. These data indicate that, in order for a genetically attenuated human malaria vaccine to be safe a number of genes governing independent biological processes all critical for liver stage development will need to be removed to ensure complete arrest of the parasite.

38

TRANSPORTER GENES OTHER THAN *PMDR1* AND *PFCRT* MAY ALTER THE RESPONSE OF *PLASMODIUM FALCIPARUM* TO LUMEFANTRINE

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The combination of Artemether (ART) and Lumefantrine (LM) is an important malaria treatment regimen in many endemic countries. Artemisinin (of which ART is a derivative) resistance has been reported and recent studies indicate that resistance to ART-LM could evolve quickly, with LM providing the main selective pressure for resistance. Strategies to overcome drug resistance require a detailed understanding of the mechanisms of resistance. For instance, studying the mechanisms of LM resistance may aid in the identification of molecular markers that can be used to monitor ART-LM efficacy. Previous studies have highlighted the important role of transporters in mediating drug resistance. As part of an exploration of the mechanisms of LM resistance, we investigated the change in the expression levels of parasite transporters in *in vitro* selected LM resistant parasites. We cultured the P*lasmodium falciparum* multidrug resistant reference strain V1S for > 1 year under LM pressure. We used the PFsanger affymetrix array to identify genes differentially expressed after LM selection. For a subset of these genes, qPCR was done to confirm microarray results. The initial LM IC50 (inhibitory concentration that kills 50% parasitaemia) of V1S was 24 nM. The resulting resistant strain, V1SLM, could grow steadily in 378nM of LM, 15 times higher than the IC50 of the parent strain V1S. Although this resistant phenotype was unstable, micro array analysis showed that 12 transporters including the multidrug resistance gene (*Pfmdr1*), multidrug resistance associated protein (*Pfmrp*) and the V-type H+ pumping pyrophosphatase 2 (*PVP2*) were differentially expressed; this differential expression was confirmed by qPCR. In addition we observed the over expression of several exportome genes on the left arm of chromosome 2 and 10 in VISLM, suggesting a deletion in the parent line. Further, gene ontology analysis revealed the over-representation of genes with transporter activity in V1SLM. In conclusion, transporters other than *Pfcrt* and *Pfmdr1* may alter *P.falciparum* response to LM. We propose further investigations in field isolates and functional studies to confirm the exact role of the 12 genes and the genes on Chromosome 2 and 10 in mediating resistance to LM and other antimalarials.

39

CCR4-ASSOCIATED FACTOR 1 IS A GLOBAL REGULATOR OF GENE EXPRESSION IN MALARIA PARASITES

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The CCR4-associated factor 1 (CAF1), a component of the major cytoplasmic deadenylase that initiates mRNA decay in eukaryotes, is highly conserved in all *Plasmodium* species. CAF1 is a part of a multi-component, gene regulatory complex in eukaryotes, termed the CCR4-NOT complex. In higher eukaryotes, the CCR4-NOT complex plays a global role in gene regulation with functions in transcription, mRNA decay and protein degradation. Genetic disruption of *P. falciparum* CAF1 results in astounding alterations in the temporal pattern of transcription and increased mRNA half-lives, which lead to mistimed expression of several proteins in the parasite intraerythrocytic stages. Premature expression of proteins controlling parasite egress from host cell, leads to an early release of unsegregated merozoites from the CAF1 disruptant schizonts and

drastically reduces the intraerythrocytic growth rate of the parasite. CAF1 is hence a global regulator of gene expression in malaria parasites essential for optimal intraerythrocytic parasite growth.

40

STRUCTURE-FUNCTION-IMMUNOGENICITY STUDIES OF PFEMP1 DOMAIN DBL2βC2 PF11-0521, A LIGAND FOR ICAM1 AND MALARIA VACCINE CANDIDATE

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We have previously identified a single PfEMP1 domain (DBL2βC2 domain of PF11_0521) from the 3D7 genome with high binding affinity for ICAM1, and showed that its semi-conserved N-terminal sequence is essential for binding. We now have prepared various point mutations in the N-terminal region as well as in Loop 4 (which was previously implicated in ICAM1 binding). We find that binding is impaired by mutations in the N-terminal sequence but not in Loop 4 (Ala to Leu, His, or Tyr), suggesting that existing *in silico* predictions of the ICAM1-PfEMP1 interaction are incomplete. Because functional antibodies that block ICAM1 binding to DBL2βC2PF11-0521 domain protect against hospitalization in our field studies, we studied antibodies raised in animals against this domain. Antibodies against E.coli-expressed truncated variants missing N-terminal sequence recognize the COS cell-expressed full-length domain but lack functional activity, while antibodies raised by DNA vaccination against full-length domain block binding. These data shed light on the requirements for PfEMP1 domain-based vaccines that might prevent severe malaria in young children.

41

A RECOMBINANT *PLASMODIUM FALCIPARUM* MEROZOITE-SPECIFIC THROMBOSPONDIN RELATED ANONYMOUS PROTEIN (MTRAP) IS A HIGHLY EXTENDED FLEXIBLE ROD LIKE PROTEIN THAT BINDS HUMAN ERYTHROCYTES

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The release of a number of *Plasmodium* genome sequences has opened up an extraordinary opportunity to discover new proteins that play crucial roles in development and invasion of red blood cells. The *P. falciparum* merozoite-specific thrombospondin related anonymous protein (MTRAP) is an example of such a novel protein. MTRAP is localized within the micronemes, then released onto the merozoite surface and processed during invasion. MTRAP has previously been shown to interact with aldolase; an actin binding protein for motility. It is hypothesized that MTRAP is released apically during invasion to mediate motility and host cell invasion. MTRAP is a cysteine rich protein with a type I thrombospondin structural homology repeat (TSR) domain. The TSR domain is a conserved domain identified in some cellular signaling proteins such as the *P. falciparum* circumsporozoite protein. We expressed, refolded and purified the full-length extracellular domain of MTRAP protein using an *Escherichia coli* expression system. rMTRAP was fully characterized by a complement of biochemical and biophysical techniques including reverse-phase HPLC analysis, atomic force microscopy, circular dichroism, sedimentation analysis, analytical size exclusion chromatography (SEC) with online multi-angle light scattering and quasi elastic light scattering, and mass spectroscopy. Our results demonstrate that purified rMTRAP appears in solution as a highly extended protein over 1 nm in width x 25 nm in length. An evaluation of whether rMTRAP bound human erythrocytes using a classical erythrocyte binding assay demonstrated that rMTRAP bound human erythrocytes. Taken altogether, MTRAPs role in erythrocyte invasion merits more investigation.

42

PLASMODIUM VIVAX RESURGENCE IN CHILDREN A DECADE AFTER MALARIA ELIMINATION ON ANEITYUM ISLAND

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Islands provide natural ecological experiments with a great potential for intervention studies. Aneityum, the southernmost island in Vanuatu, is located at the southeast edge of the malaria extension in Pacific. The implementation of a combined elimination package led to complete absence of *Plasmodium falciparum* in 1991, and *P vivax* disappeared from 1996 onwards, with the exception of two imported infections in Aneityum. We concluded that malaria can be eliminated on isolated islands with existing tools if there is a high degree of community commitment, as reported previously. One major concern is the possible resurgence due to inter-island human movement. In Aneityum interruption of malaria transmission was sustained until an epidemic of *P. vivax* was reported in early 2002. We investigated age-specific prevalence of malaria parasites during this epidemic in the context of seroepidemiological observations and molecular analysis of parasite diversity. Of *P. vivax* infections (28/1570) detected in two population-wide surveys 26 were found in individuals born after 1991. Positive antibody responses in 1998 to erythrocytic stage antigens and recombinant circumsporozoite proteins of *P. vivax* and/or *P. falciparum* were significantly lower in the population born after 1991 than in those born before 1972 (1 % vs 70% for erythrocytic and 0% vs 15% for sporozoite). Sero-conversion rate (SCR) for both parasite species on Aneityum clearly show a step in sero-prevalence indicative of the change in transmission related to elimination efforts in the past. Current SCR for *P. falciparum* (SCR 0.006, CI 0.003-0.010) and *P. vivax* (SCR 0.002, CI 0.000- 0.040) are 10-20 fold lower than pre-elimination levels (*P. falciparum* SCR 0.04, CI 0.03- 0.06 and *P. vivax* SCR 0.030, CI 0.020- 0.035). Sequence diversity of *Pvmsp1* and *Pvcsp* was very limited in Aneityum (genotype diversity h = 0.15), when compared with that on other islands of Vanuatu (h = 0.89 - 1.0), where SNPs in these antigen genes are stable. Our results suggest recently imported parasites as the probable source of this *P. vivax* resurgence in Aneityum. The persistence of antibody responses to parasites in previously exposed populations and the limited parasite gene pool are likely to have limited the age distribution of parasites to individuals born after elimination in Aneityum.

43

VERY LOW MALARIA PREVALENCE ON ISABEL IN THE CENTRAL SOLOMON ISLANDS

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The Solomon Islands Ministry of Health monitoring data derived from its Vector Borne Disease Control Program indicated very low morbidity and no malaria mortality on the island of Isabel. A comprehensive malaria survey was carried out in October 2009 by the Ministry of Health to confirm this and determine if the island was suitable for malaria elimination activities. One third of the population of Isabel (n=8600) gave their informed consent for a finger stick blood sample from which Giemsa stained blood smears, malaria serology and polymerase chain reaction (PCR) specimens were taken. Forty-nine sites involving 129 villages distributed the survey sampling across the entire island. Only a single positive blood smear for *P. falciparum* was found for a point prevalence rate of 0.012%. Following PCR testing of 2071 samples and review of blood smears from all positive PCR samples, another 2 positive smears were found. When all positive PCR samples were counted, the point prevalence rate increased to 0.193 %. Attempts to use malaria rapid diagnostic tests on febrile persons to identify malaria infections were unsuccessful due to low malaria infection rate in fever patients. Malaria serology testing suggested that transmission of *P. falciparum* markedly decreased 4 years previously and *P. vivax* 8 years previously. Ten percent of participants reported travelling out of Isabel Province from April to October 2009 indicating the potential for reintroduction of exogenous parasites. Isabel has very low malaria prevalence and is a good candidate to advance to malaria elimination. Better parasite detection methods/ algorisms are required in order to facilitate active case finding and thus parasite elimination through directed chemotherapy.

44

PROGRESS ON NATIONWIDE SCALE-UP OF MALARIA CONTROL INTERVENTIONS IN SENEGAL, 2009

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Senegal is a country of 12.5 million people where malaria is endemic with seasonal transmission. The National Malaria Control Program has scaled up nationwide the distribution of long lasting insecticide treated nets (LLINs) through free, subsidized, and market channels, intermittent preventive treatment during pregnancy (IPTp), and case management with rapid diagnostic testing followed by artemisinin-based combination therapy (ACT) of confirmed cases at public health facilities and by community health workers. Proportional morbidity due to parasitologically confirmed malaria was 5.6% in 2008, according to routine health system data. A nationwide two-stage cluster sample household survey (Malaria Indicator Survey) was conducted in October 2008-February 2009 to measure intervention coverage and parasitemia and anemia in children < 5 years. We surveyed 9,291 households in 320 clusters. We found that 60% of all households owned at least one insecticide-treated net (ITN); 23% of the general population, 29% of children < 5 years and 29% of pregnant women slept under an ITN the previous night. Of women who completed a pregnancy in the past two years, 52% had taken two doses of sulfadoxine-pyrimethamine as IPTp. Treatment was sought for one quarter of children < 5 years with fever in the preceding two weeks: 19% at a public health facility, 4% at a private provider, and 2% with a family member or traditional healer. Overall, 4.6% received an ACT, and 2.2% received an ACT within 24 hours. Of children < 5 years, 5.7% had parasitemia and 7.4% had severe anemia. Mortality in children < 5 years fell from 121 per 1000 live births for the period 2000-2005 to 85 for the period 2003-2008. Since this survey, Senegal distributed 2 million LLINs in a free nationwide campaign. Interpreting the proportion of children with fever receiving treatment is difficult as only those with positive RDT results are treated. While we have not yet met our coverage targets, the dramatic decrease in under 5 mortality suggests that the scale-up of interventions may be having a positive impact.

45

PRELIMINARY RESULTS FROM THE FIRST MALARIA INDICATOR SURVEY (MIS) IN UGANDA, 2009

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Despite the scale-up of malaria control interventions, malaria remains Uganda's leading cause of morbidity and mortality and is endemic in 95% of the country. In order to obtain national and regional estimates of outcome (e.g. malaria prevention and treatment measures) and impact indicators (i.e. anemia and parasite prevalence) and to monitor progress towards the Roll Back Malaria (RBM) 2010 targets, a malaria indicator survey (MIS) was conducted. The 2009 MIS, used a two-stage cluster sample design and was conducted during peak malaria season. A standardized household and women's (age 15-49 years) questionnaire was administered to 4,421 households in 170 enumeration areas (response rate 97.5%); children, aged 0-59 months, were tested for anemia and parasitemia. Where applicable, results were compared with Uganda's 2006 Demographic Health Survey. The percentage of households owning ≥1 insecticide treated net (ITN) increased from 16% in 2006 to 47% in 2009. The percentage of children <5 who slept under an ITN the night prior to the survey tripled from 10% to 32%. Among ITN owning households, 59% of children slept under an ITN the previous night. Similarly, the percentage of pregnant women who slept under an ITN the previous night nearly doubled from 24% in 2006 to 44% in 2009. Among ITN owning households, 77% of pregnant women slept under an ITN the previous night. The percentage of women receiving two or more doses of SP for IPTp doubled from 16% in 2006 to 32% in 2009. Of the 45% of children < 5 who had a reported fever in the previous 2 weeks, 36% received an antimalarial on either the same or day after presentation. Among children <5 tested, 10% had severe anemia (Hg<8g/dL) and 42% were parasitemic. In conclusion, there have been some significant successes in improving the coverage of lifesaving malaria interventions in Uganda. However, the burden of malaria in the country is still unacceptably high and improving access and usage of malaria control and prevention activities needs to be rapidly scaled-up in order to achieve the RBM targets.

46

CASE DEFINITION FOR MALARIA IN ENDEMIC SETTINGS: ATTRIBUTABLE FRACTION IN THE GAMBIA USING MODELING METHODS

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A correct case definition for malaria is important for measuring disease burden and an endpoint for trials. In malaria endemic regions, the frequent occurrence of asymptomatic parasitaemia makes the case definition for malaria difficult. A threshold for parasite densities can be used but needs to be defined particularly with the currently changing

levels of infection in many endemic regions. The attributable fraction (AF) i.e. the proportion of fever cases that will be eliminated if individuals were completely cleared of their parasites, can be estimated from classical estimates but this method is not very useful in settings with high proportion of asymptomatic parasitaemia. Modeling methods overcome these pitfalls. We sought to determine the AF for children aged 6 months to 10 years in an area of seasonal transmission and to estimate the sensitivity and specificity of different cutoffs for parasite density. Analysis of data from a study conducted in central Gambia. Cross-sectional surveys were carried out in 2006 and 2007 in the low and high transmission seasons. Febrile children were compared to afebrile children in terms of malaria parasitaemia and the AF was estimated using classical methods. The cumulative probabilities for parasite densities were estimated for febrile and afebrile children. Logistic regression models were compared by varying the parasite density and the best model was chosen based on the fit and plausibility. In the high transmission seasons, 3513 children were examined with a mean age was 5.04 years (SD=2.68) and 52.29% were males. There were 119(3.39%) cases of fever and 512(14.57%) with *P. falciparum* parasitaemia by thick smear (mean parasite density: 1696.54 parasites/µl, SD= 19776.88). The proportion of febrile children with detectable parasites was 40/119(33.61%) compared to 472/3394(13.91%) in the afebrile (OR=3.13, 95% CI=2.11-4.65). The AF, using the classical estimates was 0.2314 while the AF estimated from a logistic model using log of parasite density was 0.9821. A cutoff of 4000 parasites/µl had high enough sensitivity and specificity (about 70%). The models will be presented and the results obtained in the high and low transmission season will be compared.

47

ACTIVE CASE DETECTION TO TARGET RESERVOIRS OF ASYMPTOMATIC MALARIA IN A RURAL DISTRICT IN SOUTHERN PROVINCE, ZAMBIA

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In malaria endemic areas, asymptomatic infections are not uncommon with some areas reporting over half the population carrying malaria parasites. Identifying these individuals is difficult as parasite levels in many asymptomatic infected persons are low and hard to detect using microscopy. If asymptomatic infections are not identified and treated as part of ongoing control strategies, malaria can quickly resurge once control is interrupted. To investigate how best to target asymptomatic individuals, a pilot study was conducted in Choma District, a rural area in the Southern Province of Zambia from June to August 2009. The hypothesis was that in an area where malaria control strategies are implemented and transmission is low, symptomatic cases of malaria do not occur in isolation but arise from a spatially-clustered reservoir of asymptomatic infections. Each week, nurses at participating rural health centres (RHC) communicated the number of rapid diagnostic test (RDT) positive malaria cases to a central research team. During the dry season, when malaria transmission was lowest, the research team followed up each positive case reported by the RHC by a visit to the homestead. The location was obtained by GPS and all consenting residents completed a questionnaire and were screened for malaria using thick blood film, RDT, nested-PCR, and RT-PCR for asexual and sexual stage parasites. Persons who tested positive were treated with artemether/lumefantrine (Coartem®). Data were compared with a community-based study of randomly selected households to assess the prevalence of asymptomatic parasitemia in the same localities collected in September 2009. Preliminary results show that 2.3% of 87 individuals in the household of a symptomatic case were RDT positive whereas, 0.71% of the 141 participants in randomly selected households were RDT positive. Spatial data of homesteads of the index cases suggests that clusters of malaria may be present during the low transmission season. PCR assays and statistical analysis are in progress.

48

A MODEL OF THE EFFECTS OF ARTEMISININ-BASED THERAPY ON MALARIA TRANSMISSION

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Current efforts to reduce the worldwide malaria burden depend on utilizing a variety of different interventions to either kill the parasite within the human host or interrupt its transmission. In order to determine how individual-level interventions impact parasite spread, mathematical models predicting the effects of drug treatment on infectiousness are needed. We describe here a model that simulates various stages of the *Plasmodium falciparum* lifecycle and predicts how artemisinin-based combination therapy (ACT) affects *P. falciparum* transmission from humans to mosquitoes in low endemicity settings. The model includes a within-host simulation of the asexual and sexual forms of the parasite that was developed from malaria therapy data and predictions of the onset of fever. Transmission to the mosquito is a function of gametocyte densities. ACTs are assumed to rapidly kill asexual parasites and early-stage gametocytes but not affect later-stage gametocytes. Our model predicts that early initiation of ACT treatment would interrupt transmission in low-transmission settings (i.e. where R0 is near unity) if treatment levels are 50% or greater. Active case detection would be required in order to improve effectiveness of ACTs at reducing transmission in areas of higher transmission. However, due to the extremely high R0 values found in some hyper-endemic areas of Sub-Saharan Africa, treatment with ACTs alone would not be predicted to succeed in interrupting transmission there, even with active case detection. Further, we generate the first estimates of the heterogeneities in infectivity found within populations and find a wide variance in infectivity between the 5th and 95th percentiles. These results will be incorporated into a larger transmission model that allows for the simultaneous simulation of a variety of different interventions over various endemic regions.

49

A SIMPLE HAND-HELD TEST FOR RAPID DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN THE FIELD: OPTIMIZATION OF SAMPLE COLLECTION METHODS FROM LESIONS

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Initial studies were performed to identify the optimal sampling method for obtaining parasites from Cutaneous Leishmaniasis (CL) lesions for use in combination with a field usable lateral flow immunochromatographic immunoassay (dipstick) for parasite detection. The test is based on the detection of thiol specific antioxidant protein (TSA, Peroxidoxin) a highly expressed protein present in amastigotes and promastigotes of *Leishmania major* and other *Leishmania* spp. The test uses a capture polyclonal antibody to TSA in combination with a gold conjugated monoclonal antibody directed to *L. maj*or amastigotes but reactive with TSA. Sixty patients (10 per sampling method, randomly selected) ranging from 7-89 years in age were enrolled with written informed consent at a study site endemic for *L. major* infections in Central Tunisia. We compared several methods for collecting samples for the dipstick assay since the sampling

process is critical for FDA clearance. These included dermal scraping (gold standard), swab, dental broach, dermal curette, fine needle aspirate, and plastic pipette aspirate. In each case smears were prepared, stained, and graded by microscopy using the WHO scale and compared to dipstick activity graded on a photographic color scale from 0-15. The most promising method based on this preliminary data set was the dental broach, which showed high sensitivity (100% vs microscopy) identifying 7/7 microscopy positive samples and 0/3 of microscopy negative samples. It also showed a high correlation (R2= 0.86) between dipstick intensity and parasite load. The merits of the other tissue sampling methods will be discussed. Further studies to evaluate the dental broach as the sampling method for the rapid immunoassay are planned. The pairing of a rapid diagnostic assay that can be used far forward in low resource settings and rugged environments with a safe and easy to use topical treatment drug will define the future management of patients with CL.

50

A CLUSTER OF CUTANEOUS LEISHMANIASIS ASSOCIATED WITH HUMAN TRAFFICKING

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Cutaneous leishmaniasis is an infectious disease rarely seen in the United States and therefore can be a diagnostic dilemma. Determining the social and geographic context of infection is key to diagnosis and management of this disease. This study was undertaken to study the epidemiology and response to liposomal amphotericin therapy in a cluster of five patients with cutaneous leishmaniasis due to L. panamensis at a University hospital. Patients included Somali and one Ethiopian in US Border Patrol custody. All five patients came to the US by the same human trafficking route: Djibouti ◊Dubai◊Moscow◊Havana◊Quito; from Quito, by ground to the Columbian/Panamanian border where they camped out; finally, by ground to the US/Mexico border where they were detained. After routine medical care failed to adequately treat a variety of skin lesions, all five patients simultaneously presented to our institution. The patients had chronic ulcerative skin lesions at different sites (pinna, thumb, leg, foot and thighs), stages of evolution, and size (range, 1 - 8 cm). Histological examination of punch biopsies from all patients demonstrated chronic inflammatory infiltrates; one demonstrated intracellular amastigotes typical of Leishmania spp. Culture of biopsy specimens in M199 medium grew promastigotes identified as Leishmania panamensis (Viannia group) by isoenzyme analysis and PCR. Patients' lesions responded to liposomal amphotericin B dosed at 3mg/kg on days 1-7, 10, & 14. Three patients had mild, self-resolving renal failure (maximum creatinine: 1.6mg/dL). At one month, all lesions were resolved. In conclusion, we have documented a new human trafficking route associated with importation of new world cutaneous leishmaniasis to the US. Liposomal amphotericin treatment was effective. Clinicians and public health officials should be aware of this emerging infectious disease risk.

51

INACTIVATED, CELL-BASED YELLOW FEVER 17D VACCINE-SAFETY AND IMMUNOGENICITY IN ANIMAL MODELS AND RESULTS OF A PHASE 1 CLINICAL TRIAL

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The live, attenuated yellow fever (YF) 17D vaccine produced in eggs causes rare but serious adverse events, including viscerotropic disease (case-fatality rate 64%). Moreover, live 17D vaccine is contraindicated in persons allergic to eggs, pregnant and nursing mothers, immune-suppressed persons, and must be used with caution in the elderly. As a safer alternative free from these contraindications and precautions, we have developed an inactivated cell culture-based YF vaccine. The 17D strain was grown in Vero cells grown on microcarrier beads in a single-use XDR bioreactor. Virus was purified, inactivated with β-propiolactone, and adsorbed to aluminum hydroxide adjuvant. The inactivated vaccine (XRX-001) elicited high titers of neutralizing antibody in mice, hamsters, and cynomolgus monkeys. A single IM inoculation of XRX-001 resulted in antibody titers similar to those following live 17D vaccine and two doses of inactivated vaccine induced antibody titers significantly higher than live 17D. Solid protection against challenge with virulent YF virus was demonstrated after one or two doses of XRX-001. A randomized, double-blind Phase 1 clinical trial of the inactivated vaccine was conducted in 60 healthy subjects 18-49 years of age, who received two IM injections of XRX-001 at a dose of 4.4 µg or 0.44 µg or placebo on Day 0 and 21. In a parallel study 30 travelers matched for age received live YF 17D. Subjects in the XRX-001 trial were followed for adverse events, and subjects in both studies were tested for neutralizing antibodies. Results of the trials will be presented.

52

ETIOLOGY OF FEVER IN CHILDREN FROM URBAN AND RURAL TANZANIA

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Several studies have looked at the proportion of either malaria, pneumonia, diarrhea or bacteremia among fever cases in Africa but none of them at the overall spectrum of etiologies. We aimed at investigating the precise cause of fever episodes in children attending an outpatient clinic in an urban and a rural setting in Tanzania. All consenting children aged 2 months - 10 years with a temperature >38°C were recruited. A detailed medical history and clinical examination were done to identify obvious foci of infection. A blood sample was taken to perform rapid tests for malaria and typhoid, blood culture and serological and molecular analyses. All had nasal/throat swabs taken for viral molecular investigation, urine when no obvious cause was found and stools when diarrhea was present. A chest X-ray was performed when IMCI criteria for clinical pneumonia were met. Each diagnosis was assigned a probability level (high, moderate, low) on the basis of pre-defined criteria. 1010 children were recruited, 510 in Dar es Salaam and 500 in Ifakara. Preliminary results on the causes of fever of high probability were: 50% acute respiratory infection (ARI) (31% URTI, 4% bronchiolitis, 12% non-documented pneumonia and 3% pneumonia documented by X-ray), 11% malaria, 9% diarrhoea (3% rotavirus and 6% bacterial or unknown), 6% urine infection, 3% typhoid, 1% skin infection and 20% still unknown at this stage. 4% of the children had significant bacteremia, of which half were occult. 13% had more than one diagnosis (of high probability); 1% only had both malaria and pneumonia (documented or not). 104 children had a severe disease based on WHO criteria: 38% severe ARI, 36% severe malaria, 10% severe sepsis of unknown aetiology, 8% gastroenteritis with severe dehydration, 8% severe sepsis with another infection and 2% meningitis. In conclusion, these results provide for the first time an accurate picture of the respective causes of fever in African children. As expected, ARI contribute to the largest burden of disease, most of them being URTI. There was a sizeable proportion of fevers due to typhoid documented by the rapid test for most of them. Malaria confirmed to be lower than generally thought. Results of molecular analyses and serologies

will be presented and will provide further insight on the respective contribution of bacteria and viruses, a critical issue for appropriate management of fever and rational use of antibiotics.

53

PERIPHERAL BLOOD STEM CELL TRANSPLANT RELATED *PLASMODIUM FALCIPARUM* INFECTION IN A SICKLE CELL ANEMIA PATIENT

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Nosocomial *Plasmodium falciparum* (Pf) infection is rare. Most cases involve a blood transfusion from a donor who traveled to an area endemic for malaria. We report a case of peripheral blood stem cell transplant-related Pf infection in a patient with sickle cell anemia. Both recipient and donor are from Sierra Leone. At the time of the transplant, the patient had been in the United States for two years. The donor arrived in the United States three months prior to donation. The patient developed fever and chills several days post-transplant. A diagnosis of malaria was made incidentally on a peripheral blood smear. Examination of prior blood smears revealed parasitemia beginning two days earlier. Thick and thin smear had been performed on blood from the donor prior to stem cell donation and were negative for malaria parasites. To determine whether the infection represented reactivation of occult malaria in the recipient or was related to infusion of donor stem cells or other blood products, blood samples were analyzed by real-time PCR and Pf-HRP2 antigen ELISA. Pf PCR was positive in the patient one day prior to the first positive blood smear and remained positive until treatment. Similarly, Pf-HRP2 antigen ELISA was positive days prior to the first positive blood smear. ELISA testing of pre-transplant plasma from the recipient was negative. Although PCR of the donor's blood at the time of his initial evaluation was negative, ELISA testing was positive for Pf at this time. Furthermore, repeat testing one month later, on a sample obtained prior to stem cell mobilization, was positive by PCR. The donor was treated for malaria, and follow up PCR was negative. All blood transfusion donors were screened with travel questionnaires and were negative by malaria indirect fluorescent antibody tests. These results are consistent with transmission of malaria by transfusion of peripheral stem cells from an infected asymptomatic donor with a negative blood smear. Not only does this case demonstrate that malaria parasites can survive freezing in the absence of red blood cells under appropriate conditions, but it raises questions as to the most appropriate screening method for donors from malaria-endemic regions. We propose that such donors be screened with both multi-species PCR and Pf-HRP2 antigen ELISA, and that asymptomatic donors with a positive test be treated for malaria prior to donation.

54

MALARIAL RETINOPATHY IN BANGLADESHI ADULTS

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A unique spectrum of retinal signs with important clinical and prognostic significance has been well described in African children with cerebral malaria. It has not been established whether the assessment of malarial retinopathy in adult malaria using simple direct or indirect ophthalmoscopy by non-ophthalmologists has a similar significance. 170 adult Bangladeshi patients with *falciparum* malaria, 20 with vivax malaria and 20 healthy subjects were assessed by both direct and indirect ophthalmoscopy. Healthy subjects and patients with vivax malaria did not show retinal changes, whereas in patients with *falciparum* malaria indirect ophthalmoscopy revealed malarial retinopathy in 18/21 (86%) patients with a fatal course, 31/75(41%) with cerebral malaria, 16/64 (25%) noncerebral but severe malaria, and 1/31 (3%) with uncomplicated malaria. By direct ophthalmoscopy, retinopathy was missed in one patient with cerebral malaria and graded as less severe in 7. More retinal haemorrhages were found by indirect ophthalmoscopy than direct (mean difference (95%CI) 3.09 (1.50-4.68), p<0.0001). In three patients, papilloedema was found by direct ophthalmoscopy but not indirect. For both techniques there was an increase in the severity of retinopathy with increasing severity of disease, from uncomplicated to severe to cerebral malaria (p for trend p<.0001). Renal failure, acidosis and presence of moderate/severe retinopathy were independent predictors of mortality by both indirect and direct ophthalmoscopy. Direct ophthalmoscopy by the non-ophthalmologist to assess malarial retinopathy is an important clinical tool to aid diagnosis and prognosis in adults with severe malaria. Indirect ophthalmoscopy is more sensitive to detect retinal pathology in severe malaria, but provides minimal additional prognostic information in the hands of a non-ophthalmologist.

55

A RETROSPECTIVE STUDY OF SEVERE *PLASMODIUM KNOWLESI* INFECTIONS AT QUEEN ELIZABETH HOSPITAL, SABAH, MALAYSIA

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The simian parasite *Plasmodium knowlesi* is an important cause of potentially-fatal adult malaria in Malaysia. The spectrum of disease of *P. knowlesi* in tertiary referral centers has not been described. A retrospective clinical chart review of all patients diagnosed with *P. malariae* or *P. knowlesi* by microscopy or PCR from December 2007-November 2009 at Queen Elizabeth Hospital (QEH), a tertiary care hospital in Kota Kinabalu, Sabah, Malaysia. During this period, there were 324 cases of malaria and 78 (24%) were *P. malariae/knowlesi*, with 70 records reviewed. 47 (66%) patients had uncomplicated disease, while 23 (34%) had severe disease resulting in 5 (6.4%) deaths. PCR was performed in all severe cases and confirmed *P. knowlesi* in 20 and mixed *P. vivax*/knowlesi in 1. 41 of the uncomplicated cases had PCR: 34 (85%) had *P. knowlesi* monoinfection and 5 mixed with other species. In severe disease, no cases of coma were reported, 7 patients had one, and 6 had two clinical criteria for severity, with the remainder having ≥3. Those with severe disease were significantly older (56 yrs vs 36 yrs; p<0.001), with lower oxygen saturation (90% vs 97%, p=0.006), and increased thrombocytopenia (45, 0000/µl vs 66,000/µl, p=0.006). Uncomplicated disease was successfully treated with oral chloroquine (n=19), quinine (n=18) or artemether/lumefantrine (n=10), and severe disease with quinine (n=15; 3 deaths) and artesunate (n=8; 1 death). *P. knowlesi* is a major cause of malaria in QEH, Sabah, with a high proportion having severe and fatal disease.

56

ADIPOKINETIC HORMONE (AKH) TRIGGERS LIPID MOVEMENT TO DEVELOPING INTRAUTERINE LARVAE IN FEMALE TSETSE FLIES, *GLOSSINA MORSITANS MORSITANS*

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Adipokinetic hormone (AKH) is an insect neuropeptide released to mobilize nutrients. In particular, this hormone functions as a trigger

for lipolysis in the fat body. AKH is linked to periods of high activity and starvation, however little is known on the role of its during insect reproduction. Tsetse fly reproduction is unique as larval development occurs entirely within the mother's uterus, and are deposited as 3rd instar larvae that immediately undergo pupariation. This reproductive strategy limits a female to 8-10 offspring during her lifespan. Nutrients are provided to developing larvae through a modified accessory gland (milk gland), and consists specialized milk gland proteins and lipids. We examined the ability of AKH to elicit the mobilization of stored lipids from the maternal fat body to intrauterine progeny *via* the milk gland. Analysis of *G. m. morsitans* genomic databases revealed two distinct AKH sequences that vary by only a single amino acid. One gene coding for the AKH receptor (AKHR) was also identified. Transcript abundance of the *akh* and *akhr* genes increased at the end of oogenesis/beginning of embryogenesis during the first gonotrophic cycle, then subsequently decreased and remained constant. Levels of AKHR remained constitutive throughout larval development, suggesting that protein expression is not transcriptionally regulated. Localization of AKHR utilizing western blotting and immunohistochemistry revealed this receptor is present on the tsetse fat body and larva. *In vitro* analysis of the fat body indicated lipids are released by this tissue following AKH exposure. Injections of AKH into pregnant females increased lipid levels in the hemolymph, and daily injections increased the lipid content within the larva compared to insect saline injections. Based on these results, AKH is a candidate hormone, functioning alone or in combination with other hormones, for signaling lipid mobilization from the fat body to the milk gland to feed the developing larva in pregnant female tsetse.

57

ANALYSIS OF THE PROMOTER AND REGULATORY REGION OF THE TSETSE FLY (*GLOSSINA MORSITANS MORSITANS*) MILK GLAND PROTEIN GENE (GMMMPG): THE SEARCH FOR MECHANISMS REGULATING MILK GLAND AND PREGNANCY SPECIFIC GENE EXPRESSION

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The tsetse Milk Gland Protein (GmmMGP) is an essential component of nourishment during intrauterine larval development. Transcription of gmmmgp is regulated in tight corellation with larval development. The aim of this work is to identify the regulatory sequence responsible for spatial/temporal regulation of gmmmgp expression. Regulatory region localization was accomplished via generation of transgenic Drosophila with promoter/reporter constructs utilizing 2 kB and 0.5 kB fragments of the 5' upstream sequence from the gmmmgp gene transcription start site. Transgenic analysis of the gmmmgp regulatory region in Drosophila demonstrates that the 0.5 kB region of the 5' upstream is capable of tissue and sex specific reporter expression within the Drosophila accessory gland (ortholog to the tsetse milk gland), the paraovaria. In silico identification of transcription factor binding sites was performed using MatInspector. Tsetse transcription factor homologs were identified from cDNA and genomic libraries via BLASTx search. 21 putative transcription factor binding sites were identified within this region representing 11 transcription factor families. Tsetse homologs were identified for 8 families, 4 of which have representative homologs specific to a fat body/milk gland cDNA library. Comparative analysis of the 0.5 kB gmmmgp regulatory region with 0.5 kB upstream sequence of 4 other milk gland/pregnancy specific genes (gmmtsf, gmmmgp2-1, gmmmgp2-2 and gmmmgp3) predicted binding sites for the transcription factor families of caudal, dorsal and paired homoeodomain factor. This work identifies and characterizes the upstream regulatory sequence required for tissue/sex specific expression of the tsetse gmmmgp gene and identifies putative cis-regulatory elements critical for this expression pattern. These results demonstrate the conservation of cis regulatory elements between Drosophila and tsetse and validate the use of a Drosophila model system for genetic analyses of tsetse gene transcription.

58

ABDOMINAL CONTRACTIONS DRIVE EXTRACARDIAC HEMOLYMPH CIRCULATION IN THE MOSQUITO HEMOCOEL

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Hemolymph circulation in mosquitoes is primarily controlled by a dorsal vessel that runs underneath the dorsal midline and is subdivided into a thoracic aorta and an abdominal heart. Wave-like peristaltic contractions of the heart alternate in propelling hemolymph in anterograde (toward the head) and retrograde (toward the tip of the abdomen) directions, where it empties into the hemocoel at the terminal ends of the insect. During our analyses of hemolymph propulsion in *Anopheles gambiae*, we observed that the ventral abdomen periodically contracts and hypothesized that these contractions facilitate extracardiac hemolymph propulsion in the abdominal hemocoel. To test this, we devised methods to simultaneously analyze both heart and abdominal contractions, and to measure hemolymph flow in the mosquito hemocoel. Qualitative and quantitative analyses revealed that the ventral diaphragm periodically contracts in a peristaltic manner, initiating each contraction at the thoracico-abdominal junction and propagating them toward the tip of the abdomen. All ventral abdominal contractions occur in the retrograde direction and correlate with anterograde heart contractions. To test whether abdominal contractions potentiate extracardiac hemolymph flow, we intrathoracically injected fluorescent microspheres and tracked their trajectory through the hemocoel. Quantitative measurements of microsphere movement in extracardiac regions of the abdominal cavity showed that, during periods of abdominal contractions, hemolymph flows in dorsal and retrograde directions at a higher velocity and with greater acceleration when compared to periods without abdominal contractions. In summary, these data show that abdominal contractions potentiate extracardiac retrograde hemolymph flow in the abdominal hemocoel during periods of anterograde heart flow. The physiological implications of these findings on immune competence and pathogen migration through the hemocoel will be discussed.

59

LABORATORY STUDIES ON THE COLOR PREFERENCES OF *AEDES POLYNESIENSIS* MOSQUITOES: COLOR SELECTION FOR THE DEVELOPMENT OF AN INSECTICIDE IMPREGNATED LETHAL TARGET

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Long lasting insecticide treated materials are showing great effectiveness as tools for reducing *Anopheles*-transmitted malaria. A modification of this approach may also prove to be a valid intervention in reducing the transmission of lymphatic filariasis (LF) in regions of the South Pacific where the *Aedes polynesiensis* mosquito is the primary vector. In lieu of using insecticide-treated bed nets we propose the use of insecticide impregnated outdoor visual resting targets (lethal targets; LT) better suited to control this exophilic, day-biting mosquito. These targets could be placed in strategic locations throughout communities were LF transmission is endemic and thus serve as an adjunct control along with the current method of disease control in the South Pacific; mass drug administration of DEC and Albendazole. In order to achieve the aim of impacting *Aedes* mosquito populations it is essential that the target color be attractive to *Ae. polynesiensis* mosquitoes. It is also vital that the color and pattern of the target be acceptable to the local community in order to maximize uptake of the resulting product. Here we present results showing comparative mosquito attractiveness for six different potential targets using a novel photographic-based small cage attractiveness assay system

developed in our laboratory. Mosquito landing behavior and resting behavior on targets of interest paired with an adjacent white control target was measured using the ImageJ software program. Comparisons were made between *Ae. polynesiensis* females of different ages as well as between female and male *Ae. polynesiensis* mosquitoes. *Ae. polynesiensis* mosquitoes displayed significant differences in terms of their preference for different colored targets. Future tests will need to evaluate how insecticide impregnation of the targets will affect *Ae. polynesiensis* response in both small cage and semi-field cage conditions. The results of this study demonstrate the promise of this approach for vector control in the South Pacific.

60

SAND FLIES DEFENSIN: EVOLUTIONARY AND PHYSIOLOGY ASPECTS

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Characterization of insect's antimicrobial peptides (AMP) is important in attempt to find novel molecules that could act against vector-borne diseases. This includes not only the identification of the gene itself but also biochemical features and the expression regulation of such AMP under several different situations naturally faced by the insect vector. Considered by WHO as one major neglected disease, the leishmaniasis is transmitted by its vector, the sand flies. We are presenting the characterization of defensin, the only sand flies AMP identified to date, from several different species from New and Old World, including the full DNA sequence of the *Lutzomyia longipalpis* defensin also the intron, 5' and 3' UTR regions. We established some aspects of this AMP like the molecular evolution in the Phlebotominae subfamily and analyze the putative occurrence positive selection. We identified differences in the expression profile of New and Old world sand flies during the developmental stages and observed a defensin positive modulation in *Leishmania chagasi* infected *L. longipalpis* and a negative regulation after a blood meal. We also analyze the expression profile of male and female challenged *L. longipalpis.* The orally challenges from a Gram + and Gram - bacteria result in little modulation of the defensin expression, but the sugar feeding result in the increase of the AMP transcription. It would be interesting to further analyze the differences from Old and New World sand flies defensin including a putative gene duplication as observed in some mosquitoes, this issue will be more clear after the release of sand flies genomes. As future goals is the evaluation of any leishmanicidal activity of the sand fly defensin. The characterization of the AMP physiology roles in the insect biology would give more tools in the development of new strategies in vector-borne diseases controls.

61

EVIDENCE OF PRE COLUMBIAN TUNGIASIS IN AMERICA

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Tungiasis is an ancient parasite that originated in America and was later transported to the Eastern Hemisphere on transatlantic voyages in the nineteenth century. Although it was first documented by Spanish chroniclers after the arrival of Christopher Columbus in the New World, little is known about its presence in pre-Hispanic America. The Inca Empire (A.D. 1430-1532), the vast pre-Columbian civilization of South America, was one of the most evolved cultures at the time of the discovery of the Americas. Several diseases, including tungiasis, were represented in clay potteries, jars or ceramics (called huacos) by their predecessors, tribes that occupied the coastal area of modern Peru. In view of the scarcity of documentation of the presence of tungiasis in Peru, we conducted a retrospective search that included the appraisal of written evidence such as ancient manuscripts and later books by chroniclers, travelers, entomologists, naturalists and anthropologists; doctoral theses; ephemeral journals, periodicals, and pamphlets in the original Spanish, French and English; and assessment of earthenware representations during selected visits to storage facilities in museums in Peru and in the US. We used all available local (n=35) and scientific names (n=9) ascribed to *Tunga* spp. over the last four centuries. To date, 4 anthropomorphic figures representing pre-Hispanic tungiasis have been identified: two anthropomorphic globular potteries, and a single-spout bottle, all from Chimu Culture (c. A.D. 1200-1470); and a new, never described or reproduced fragment from Maranga Culture (c.A.D. 150-650), with pathognomonic lesions of tungiasis. Tungiasis is an old disease that has been endemic to indigenous Peruvians for centuries, a fact that can be illustrated by anthropomorphic vessels with pathognomonic lesions in diverse stages of evolution. Our new photographed fragment is the fourth representation of *Tunga* spp. known to date in pre- Columbian American art and the only vessel that depicts different stages of tungiasis, thus representing an explicit evidence of its of its endemicity among Ancient Peruvians. Identification and analytical evaluation of these anthropological pieces dispersed now among numerous museums worldwide are of paramount importance to understand the history and impact of this flea that continues to affect Peruvians as it did in pre-Incan times

62

RNAI SILENCING OF *PHLEBOTOMUS PAPATASI* MIDGUT MOLECULES AND EFFECTS ON *LEISHMANIA MAJOR* DEVELOPMENT

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For a successful development within the midgut of the sand fly vector, *Leishmania* must overcome several barriers which are imposed by the vector that include an early proteolytic attack, the need to escape the peritrophic matrix (PM), and attachment to the midgut epithelia to prevent excretion with the remnants of the blood meal. The ability to overcome these barriers has been associated with species specificity, and interference with the sand fly vector-parasite balance can change the outcome of the infection in the the vector. Leishmania lipophosphoglycan (LPG) was shown to be a critical molecule in the midgut attachment process for some sand fly-Leishmania pairs. Further, blockage of a midgut LPG receptor, PpGalec, severely impaired *L. major* development and survival in the midgut of *Phlebotomus papatasi*. These results supported the use of transmission blocking strategies against sand fly-transmitted leishmaniasis (TBV). Following overall analyses of the midgut transcriptome of the sand fly *P. papatasi*, several midgut molecules were selected as potential TBV candidates based on their response to infection with *Leishmania major*. We are investigating some of these targets, which include a midgut-specific, blood induced chitinase (Ppchit1) previously characterized, three novel peritrophins (PpPer1-PpPer3) and several proteases. Analyses of expression profiles of the peritrophins revealed that PpPer1 and PpChit1 are only expressed in midguts whereas PpPer2 and PpPer3 are also expressed in the hindgut and malpighian tubules, respectively. PpChit1 is presumably involved in the formation/maturation of the PM in the gut of the sand fly after a blood meal. As we predicted, knockdown of PpChit1 via RNAi led to a significant reduction of *L. major* within the gut. In contrast, knock down of PpPer1 led to an increase in the parasite load. One possibility for the effect detected for PpPer1 is that its knockdown increases the escape of the parasite from the PM. Another option could be related to an increase in the availability of nutrients to the Leishmania due to greater influx of digestive proteases towards the blood bolus. These results will likely bring new understanding of underlying events involved in the cycle of Leishmania within the sand fly vector. These and other issues related to the RNAi-induced phenotypes detected will be discussed.

63

PREDICTING THE CHANCES OF ELIMINATION IN PARASITE INTERVENTION PROGRAMS

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Large-scale intervention programs to control or eliminate several neglected tropical diseases are underway worldwide, yet stopping points for these programs remain unclear. Recent epidemiological work has highlighted how the heterogeneity inherent in the transmission dynamics of macroparasites can result in elimination thresholds varying between local communities. We examine the empirical evidence for this hypothesis and its implications for the global elimination of the major macroparasitic disease, lymphatic filariasis, by applying a statistical procedure, named Bayesian Melding, to fit a dynamic model of the transmission of this parasitic disease to field data from nine villages with different ecological and geographical characteristics. Baseline lymphatic filariasis microfilarial age-prevalence data from three geographically distinct endemic regions were used to fit the relevant filariasis transmission models. We then examined elimination thresholds implied by each of the datasets to evaluate site-specific heterogeneity in the values of these thresholds and investigate the ecological factors that underlie such variability.

We also applied 5 rounds of simulated mass drug administration (MDA) to the model and compared model predictions of the likelihood of elimination, or infection re-emergence, with longitudinal follow-up data from Papua New Guinea, where the population was monitored during 5 rounds of MDA and then 10 years after the final treatment round. Model parameters relating to immunity, parasite establishment, and parasite aggregation, varied significantly between the nine different settings, contributing to varying parasite elimination thresholds. The probability that the parasite will be eliminated following 5 rounds of MDA decreases markedly but non-linearly as the Annual Biting Rate and parasite reproduction number increases. We also discuss the possibility that reintroduction of infection through immigration may be occurring and thwarting elimination efforts.

64

LONG-TERM IMPACT OF REPEATED MDA ON LYMPHATIC FILARIASIS DISEASE IN PAPUA NEW GUINEA

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In addition to breaking transmission, a goal of the Global Program to Eliminate Lymphatic Filariasis (GPELF) is to ameliorate clinical pathology due to *Wuchereria bancrofti* infection. Prior observations in a highly endemic area of Papua New Guinea showed that the prevalence of leg lymphedema and hydrocele was reduced during a 5-year mass drug administration trial, as reported previously. However, the long-term impact of MDA on clinically overt LF morbidity has not been established. We investigated the possible resurgence of LF morbidity 10 years after 5 annual rounds of MDA were given from 1994 to 1998. Physical examinations were performed on all persons >1 year old; the presence and severity of hydrocele and lymphedema of the extremities and breasts were graded according to WHO criteria as before. In communities where pre-MDA transmission was moderate, hydrocele prevalence decreased from 12% to 3% after 5 years after MDA began ($p<0.001$) and remained low (1%, $p=0.194$) 10 years after no additional LF interventions. In communities where pre-MDA transmission was high, hydrocele prevalence decreased from 27% to 10% ($p<0.001$) 5 years after MDA began but increased to 20% ($p=0.021$) 10 years later. For leg lymphedema, the most common disease site after hydrocele, 52% (14/27) and 44% (8/18) of residents of moderate and high transmission communities with chronic leg pathology during the MDA trial no longer had disease 10 years later. Less than 2% of 2474 individuals examined had newly diagnosed leg lymphedema. Transmission as measured by landing catches of infective anopheline vectors was ongoing but reduced at all sites 10 years after cessation of MDA. These data support hypotheses that the community-wide disease reversal effect of MDA may be attributable to reduction in both established infections and decreased exposure to mosquito-borne infective larvae.

65

SELECTING THE DIAGNOSTIC TOOL TO DEFINE THE ENDPOINT OF PROGRAMS TO ELIMINATE *WUCHERIA BANCROFTI*

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Successful mass drug administration (MDA) campaigns have brought several countries near the point of Lymphatic Filariasis (LF) elimination. A diagnostic tool is needed to determine when the prevalence levels have decreased significantly such that MDA campaigns can be discontinued without the threat of recrudescence. This tool needs to be highly specific, as the proportion of false positive results will increase as the true prevalence of infection declines. Antigen detection by either the ICT Card Test or Og4C3 ELISA assay has been considered the best approach to detecting this low level of *W. bancrofti* infection. The objective of this study was to determine which of these two tests is better suited for detecting the endpoint of the elimination program. An eight-country study was conducted assessing the performance of eight diagnostic tests, including Bm14, PanLF, *Brugia* Rapid, Urine SXP, ICT, Og4C3, Blood Smear, and PCR on a panel of 9,884 patient specimens. ICT and Og4C3 tests detected similar levels of antigen prevalence, 9.7% and 10.8% respectively. The specificities of the two tests were also similar (Og4C3 sp=93%, ICT sp=92%), while the sensitivity of the Og4C3 was significantly better than the ICT (Og4C3 se=87%, ICT se=76%). A closer look at the ICT/Og4C3 discordant pairs suggests that ICT test may be capturing a large number of false positives, relative to Og4C3. Adopting a test-retest strategy with the ICT test can greatly improve the specificity of the ICT test relative to Og4C3. Under such a strategy, an initial positive ICT result would be considered 'indeterminate' until a second confirmatory ICT test is conducted. This test-retest method can reduce the ICT false positivity rate by 92%, increasing the specificity of the ICT test to 99%. Because the Og4C3 test is lab-based, it does not easily lend itself to such a strategy. Therefore, we recommend that countries employ this test-retest strategy with the ICT card test to assess whether or not the threshold for stopping MDA has been met.

66

EPIDEMIOLOGIC ANALYSIS OF LYMPHATIC FILARIASIS DIAGNOSTIC ASSAY CHARACTERISTICS FOR MONITORING ELIMINATION

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Existing tools for lymphatic filariasis (LF) detection have been optimized for mapping and prioritizing areas that are targets for elimination programs using mass drug administration (MDA). However, the value of these tools for monitoring progress towards transmission elimination has not yet been established. This study evaluates human blood samples from a ten year follow-up of five annual MDAs in Papua New Guinea to quantify the population-level operating dynamics of four diagnostic assays: microscopy for blood microfilaremia (MF), parasite DNA, filarial antigen, and IgG4 antibody against BM14 across moderate and low transmission intensities. Sensitivity and specificity of the tools relative to MF were respectively 100% and 66% for antibody against BM14, 91% and 80% for filarial antigen, and 82% and 96% for parasite DNA. Whereas sensitivity and negative predictive value for these tests were high regardless of transmission intensity, filarial antigen and IgG4 antibody tests were 1.6 and 2.4 times more specific in areas of low relative to high transmission. Assays for DNA remained sensitive and specific (>70%) across a range of transmission indices. Considering children under 10 years who were born after completion of MDA revealed a pattern of biomarkers such that positivity by antigen and parasite DNA were 89% and 92% percent lower than IgG4 antibody to BM14. The variations in these assays are likely related to varying levels of transmission, limits of detection of MF, and differing uptake and decay mechanisms of the biomarkers. These results suggest that combinations of various diagnostic tools will be optimal for monitoring and potentially verifying local elimination of LF.

67

A FIELD-TESTED, POST-MDA APPROACH FOR LYMPHATIC FILARIASIS PROGRAMS

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WHO guidelines for elimination of lymphatic filariasis (LF) using mass drug administration (MDA) are well established, and guidelines for stopping MDAs are currently being reviewed. Less defined are the steps to take once MDAs are interrupted. We developed a post-MDA approach that includes (1) a sustainable, nation-wide LF surveillance system, (2) nation-wide re-mapping, and (3) active follow-up of cases identified by these methods. We piloted this approach in Togo with initiation of surveillance in 2006 and national remapping in 2010. The LF surveillance system tests hospitalized patients for microfilaria. During its first two years, 8050 persons were tested and two were positive. Follow-up of these cases found no evidence of ongoing transmission. In 2009, we evaluated this system. Patients tested resided in 1214 distinct villages or cities, and in all 35 districts of the country. Plotting these villages revealed that some remote parts of the country were under-sampled. We therefore adapted the system to include collection of filter-paper blood spots, which are sent to Lomé for Og4C3 ELISA, at health posts in these regions. In considering testing of donated blood as an alternate approach, we plotted the national blood bank's catchment area. This revealed that most blood donors reside near the main urban donation centers. Of the 9438 donors whose blood was screened in Lomé in 2008, over 7800 (83%) live in Lomé or its immediate outskirts. This suggests that screening of donated blood may be an inefficient tool for LF surveillance.

To ensure that no undetected foci of endemic transmission remain in Togo, we repeated national mapping. Because 30-cluster surveys were conducted in previously endemic areas in 2008, re-mapping was only undertaken in districts considered non-endemic during the initial mapping (done in 2000). Villages were selected by randomly placing a 35 km2 grid over the country; additional villages were selected in areas with high LF morbidity and along national borders adjacent to endemic areas of neighboring countries. In total, 7600 persons in 76 villages were tested by rapid ICT test. Data from this mapping will be presented.

Countries nearing elimination of LF cannot afford to lose their investment by allowing re-introduction or recurrence of LF post-MDA. We present here a field-tested approach that can serve as a model for post-MDA activities in developing countries.

68

HAS INTERRUPTION OF *SIMULIUM NEAVEI S.S.* TRANSMITTED ONCHOCERCIASIS BEEN ATTAINED IN THE KASHOYA-KITOMI FOCUS?

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Uganda announced a new policy of onchocerciasis elimination in early 2007. The strategy for elimination includes increasing ivermectin administration to twice per year, and vector elimination activities through ground laviciding. The effort to eliminate *Onchocerca volvulus* in the Kashoya-Kitomi focus in western Uganda began that same year. The focus covers about 339 km2 with "at risk" population of about 200,000 people. Annual ivermectin treatment has been offered in that focus for 16 years. Some experimental larviding was done in 2003. 1991 and 2005 baseline data were available for *Simulium neavei s.s.* infection, and 1991 and 2003 baseline data for human infection (skin snips). Before beginning larviciding in 2007, *Simulium* adult fly collection was established at 6 sites, and crabs were trapped and analysed for infestation of *S. neavei* larval stages. Abate larviciding began at 63 sites during May, 2007. Mass treatment with an annual dose of ivermectin had started in 1992, and twice yearly treatment launched in 2007. The focus attained at least 90% in every round of treatment every year for the last three years. In 1991, Community microfilarial load (CMFL) was 21 mf/skin snip. In the same year, *Simulium* flies density was 500 fly man hour (FMH), fly infection rate (all larval stages) above 50%, and fly infective rate (L3 larvae) at 10%-12%. In 2005, fly density was 72 FMH (probably decreased due to experimental larviciding), fly infection, 14.2%, fly infective rate, 3.2%. CMFL in 2003 was 6.4. With full implementation of the elimination policy, *Simulium* fly abundance was reduced to less than 10 FMH since December, 2008. Crab infestation with *S. neavei* has decreased from 40.5% in May, 2007 to less than 1% by February 2008, and zero for the last quarter of 2009. Interruption of onchocerciasis transmission may have been attained, but new results from skin snips in the population, and Ov16 antibody in children are required to determine if all interventions can be halted.

69

IMPACT ASSESSMENT OF REPEATED ANNUAL IVERMECTIN ON OCULAR AND CLINICAL ONCHOCERCIASIS 14 YEARS OF ANNUAL MASS DRUG ADMINISTRATION IN EIGHT SENTINEL VILLAGES, SOUTHEAST NIGERIA 2008

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We conducted a cross sectional survey in 2008 after 14 annual rounds of mass drug administration (MDA) with ivermectin to measure the impact on clinical onchocerciasis. We studied eight sentinel villages in southeast Nigeria, for which we have baseline parasitological data from 1995. During the survey we 'skin snipped' 940 consenting participants to determine microfilaria (mf) prevalence and community microfilaria load (CMFL); we also examined 839 persons by slit lamp for evidence of ocular onchocerciasis. We found a significant (76%) decrease in mf prevalence in all villages (62.43% in 1995 compared to 14.72% in 2008) as well as an 89% decrease in CMFL (2.1mf/gm in 1995 compared to 0.23mf/gm in 2008). Both findings were significant (P<0.001). *Onchocerca*l nodule prevalence also decreased significantly in all the villages. We observed 2% overall punctate keratitis that could have been attributable to onchocerciasis. These observations show that onchocerciasis is no longer a public health problem in the sentinel villages. However, we found 14.7% of 102 children below 10 years had mf in their skin snips, suggestive of continued acquisition of onchocerciasis infection during the 14 year treatment period. Treatment coverage in most of the villages were <80% (eligible population) with occasional omitted rounds, which likely contributed to continued transmission. We conclude that ivermectin treatment needs to continue, and if elimination is contemplated, enhanced programmatic support is needed to increase coverage, and twice per year treatment should be considered.

70

PLANTS USED BY TRADITIONAL HEALTH PRACTITIONERS OF NATORE AND NAOGAON DISTRICTS, BANGLADESH TO TREAT DIABETES MELLITUS

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Diabetes mellitus (DM), particularly Type 2 DM is estimated to affect over 05 million people in Bangladesh alone. This ailment is spread world-wide and particularly in the developed countries. Modern allopathic medicines cannot cure the disease but only aids in lowering blood sugar levels, which is a particular characteristic of this ailment. DM is mostly treated in the rural areas of Bangladesh by traditional health practitioners (THPs). The common form of treatment is with plants, the usage of which varies from district to district and even can vary considerably between THPs. As part of our ongoing program to complete an ethnopharmacological survey of Bangladesh, we undertook a survey amongst the THPs of Natore and Naogaon districts, which lie in the central-north region of the country. Interviews were conducted of the THPs and detailed information obtained as to plants, parts of the plant used, formulation, mode of preparation, and dosages. Plant samples were collected and pressed in the field and identified at the Bangladesh National Herbarium. 04 were found to be used by the THPs of Natore district to treat DM. These plants (with plant parts used given in parenthesis) included *Mangifera indica* (fruit), *Coccinia cordifolia* (leaf), *Lawsonia inermis* (leaf), and *Cynodon dactylon* (leaf). The THPs of Naogaon district uses 06 plants to treat DM. These plants included *Catharanthus roseus* (leaf, flower), *Alocasia macrorrhizos* (all-parts), *Coccinia cordifolia* (all-parts), *Kalanchoe pinnata* (leaf), *Mentha spicata* (leaf), and *Scoparia dulcis* (all-parts). *Cynodon dactylon*, and *Catharanthus roseus* are also used in the alternative medicine systems of Mexico and South Africa; respectively, to treat DM. Scientific studies have established the hypoglycemic potential of *Mangifera indica*, *Coccinia cordifolia*, *Lawsonia inermis*, *Cynodon dactylon*, *Catharanthus roseus*, and *Scoparia dulcis*. It is important to conduct more studies towards elucidation of the pharmacological constituent(s) responsible for the hypoglycemic activity and evaluate their potential in the treatment of DM.

71

AN ETHNOPHARMACOLOGICAL SURVEY OF JAMALPUR SADAR AREA, JAMALPUR DISTRICT, BANGLADESH USED FOR TREATMENT OF "HARD TO CURE" DISEASES

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Jamalpur district is in the north-central region of Bangladesh. The predominantly rural population of the district relies on folk medicinal practitioners, otherwise known as Kavirajes for treatment of their various ailments. We conducted an ethnopharmacological survey among the Kavirajes of Jamalpur Sadar area in this district with the objective of collecting information about the medicinal plants used by the local Kavirajes. A semi-structured questionnaire was used for the interview and Kavirajes pointed out medicinal plants during field-walks and described their uses. Medicinal plant specimens were identified at the Bangladesh National Herbarium. The various medicinal plants used by the Kavirajes (with ailments treated given in parenthesis) included *Cestrum nocturnum* (to stop bleeding), *Lannea grandis* (dog-bite, low sperm count), *Heliotropium indicum* (cataract), *Citrus grandis* (to increase appetite, blood purifier, fever), *Syzygium cumini* (tooth infection, dysentery, diabetes, kidney stones), *Ficus hispida* (dysentery), *Streblus asper* (debility), *Curcuma longa* (allergy), *Typhonium giganteum* (kidney stones, to stop bleeding), *Terminalia belerica* (asthma, allergy, to maintain heart, lungs & liver in good condition), *Parthenocissus quinquefolia* (edema), *Spilanthes acmilla* (infections on the head), *Litsea glutinosa* (low sperm count), *Caesalpinia bonduc* (menstrual problems, infertility in women, to expedite childbirth), *Achyranthes aspera* (jaundice), *Justicia adhatoda* (coughs, asthma, menstrual problems, jaundice, hepatitis B), *Calotropis gigantea* (asthma, pneumonia), *Cassia alata* (scabies), *Averrhoa carambola* (dandruff), *Cucurbita maxima* (gastrointestinal problems, joint pain, colds, constipation, piles), *Costus speciosus* (erectile dysfunction, low sperm count), *Cissus quadrangularis* (bone fracture), and *Terminalia chebula* (bloating, gastrointestinal disorders, stomachache, heart disorders, debility, helminthiasis). The medicinal plants warrant further scientific studies as potential sources of pharmacologically active compounds for treatment of diverse ailments.

72

APPLYING A KNOWLEDGE-TO-ACTION FRAMEWORK FOR PRIMARY PREVENTION OF SPINA BIFIDA IN TROPICAL AFRICA

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The objective of this study was to increase capacity for primary prevention of spina bifida through folate supplementation in the Democratic Republic of Congo (DRC), using a knowledge-to-action methodology. The design was a mixed quantitative and qualitative methods: surgical case series,

survey questionnaires (knowledge, attitudes and practices), focus group discussions (FGDs), video media evaluation (satisfaction and knowledge gain questionnaires). HEAL Africa hospital in eastern DRC, where resource limitations and threats to human security contribute to restricted capacity for the management and prevention of congenital malformations. Participants included women of reproductive age, families affected by spina bifida, and community members. A case series of 27 patients undergoing surgery for spina bifida demonstrated a short tem mortality of 15% and long-term disability in survivors. Qualitative data revealed an additional heavy psychosocial burden of illness. A survey of knowledge, attitudes and practices demonstrated a low level of folate awareness (53%) among women of reproductive age. FGDs revealed exotic etiologic views, significant gender issues, and several barriers to folate use. A culturally tailored radio broadcast and an educational video were designed and produced locally based on qualitative and quantitative findings. Evaluation of the video documented high levels of viewer satisfaction and unequivocal knowledge gain ($p<0.001$). In conclusion, spina bifida poses a significant burden on affected patients and their families in the African context, but folate is under-utilized as a prevention strategy. Patient education through video media results in increased awareness and understanding of spina bifida and folate, a first step in empowering women to reduce the risk of spina bifida in their children in the absence of population-wide food fortification.

73

HOME AND COMMUNITY MANAGEMENT OF MALARIA AND PNEUMONIA IN CHILDREN UNDER FIVE: A CLUSTER RANDOMIZED TRIAL OF AN INTEGRATED APPROACH

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Malaria and pneumonia are major causes of mortality in children under-five in Uganda. Most children receive care first from drug shops which sometimes have untrained providers, expired drugs and give under doses. Distribution of pre-packed antimalarials by community medicine distributors (CMDs) has been shown to reduce under five mortality. Pneumonia was not catered for at CMD level and children with pneumonia were often not taken to health facilities when referred. This study was undertaken to determine the impact on under-five mortality achievable through distribution of pre-packed antimalarials (Coartem) and antibiotics (Amoxicillin) by CMDs for presumptive treatment of malaria and pneumonia in children 4-59 months. Febrile children in nine intervention parishes are given pre-packaged Coartem and pre-packed Amoxicillin when they present with a high respiratory rate. In nine control parishes, febrile children are given pre-packed Coartem and those with high respiratory rate are referred to health facilities. The proportion of febrile children treated by CMDs and adherence to treatment has been assessed. After three months of implementation, 10.7% of those who sought treatment outside the home got it first from the CMDs, 13.4% from government health facilities, 35.7% from drug shops, 35.7% from private clinics and 4.6% from general shops or neighbours. There was no significant difference between intervention and control areas. A total of 208 caretakers who had received treatment from CMDs were assessed, 97.6% (203/208) took all the Coartem given to them and the other five were saving it for later use. Of the 49 children who had taken Amoxicillin, 11/49 (22.4%) did not take all the tablets given and 5/11 stopped because the child got better. Utilization of CMDs is still low but adherence to treatment especially for Coartem is high. More behaviour change communication to improve utilization of CMDs needs to be done and non-completion of doses needs to be strongly discouraged as it brings drug resistance and children may not get cured.

74

BRUCELLA - A GREAT IMITATOR

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Brucellosis is an important re-emerging bacterial zoonosis and class B bioterrorist agent. The timely diagnosis is a challenge to clinicians because of its wide spectrum of manifestations and slow growth rate in blood cultures. This retrospective study was carried out in a tertiary care center in Southern India. 68 patients with brucellosis in the last five years were studied with reference to symptoms, occupation, epidemiology, treatment, complications and outcome. Of the 68 patients, 46 (67.64%) were males, 22 (32.35%) females in the age group of 9-75 years. 44 (64.70%) had history of contact with domestic animals. 44 (64.70%) presented with fever of <60 days, and 24 (35.3%) >60 days. The symptoms included fever in 68 (100%), arthralgia 23 (33.82%), myalgia 21 (30.88%), headache 16 (23.52%), gastrointestinal symptoms 19 (27.94%) and altered sensorium in 3 (4.41%) patients. HBsAg was +ve in 8 (11.76%), HIV +ve 2 (2.94%), steroid therapy 3 (4.41%), type 2 diabetes mellitus 13 (19.11%) and alcoholism in 10 (14.70%) patients. Labs showed anemia in 39 (57.35%), leucocytosis 10 (14.70%), leucopenia 10 (14.70%), monocytosis 43 (63.23%), thrombocytopenia 23 (33.82%), thrombocytosis 2 (2.94%), high ESR 55 (80.88%), elevated transaminases 26 (38.23%), elevated alkaline phosphatase 16 (23.52%) and ultrasound (hepatospenomegaly) in 24 (35.29%) patients. *Brucella* species (BACTEC) was grown in 50 (73.52%), brucella agglutination test titers >1:320 in 61 (89.70%), widal positive 14 (20.58%), endocarditis 2 (2.94%), bone marrow granuloma 2 (2.94%), bone marrow culture growing brucella 3 (4.41%) and CSF culture with brucella in 2 (2.94%) patients. Complications were meningitis 2 (2.94%), carditis 2 (2.94%), orchitis 2 (2.94%), musculoskeletal 2 (2.94%) and death in 2 (2.94%) patients. Treatment-6 (8.82%) patients received empirical antitubercular therapy, 48 (70.58%) doxycycline and aminoglycosides, 17 (25%) doxycycline and rifampicin and 3 (4.41%) doxycycline, rifampicin and aminoglycosides. There were three conclusions: 1. Brucellosis is misdiagnosed as enteric fever or tuberculosis in endemic areas; Physicians must consider brucellosis in prolonged febrile diseases. 2. Risk factors are diabetes mellitus and immunocompromised states; 3. Early diagnosis and treatment can prevent morbidity and mortality; and 4. Effective control measures should be instituted in developing countries.

75

ASSESSING THE IMPACT OF TOPOGRAPHY ON MALARIA EXPOSURE AND MALARIA EPIDEMIC SENSITIVITY IN THE WESTERN KENYA HIGHLANDS

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The sensitivity of a site to malaria epidemics depends on the level of immunity of human population. This study examined how terrain in the highlands affects the exposure and sensitivity of a site to malaria. The study was conducted in five sites in western Kenya, two U-shaped valleys (Iguhu, Emutete), two V-shaped valleys (Marani, Fort-Ternan) and one plateau (Shikondi) for ten months among 6-15 years old children. Exposure to malaria was tested using circum-sporozoite protein and merozoite surface protein immunochromatographic antibody test; malaria infection was tested by microscopic examination of thick and thin smears. The mean antibody prevalence was 20.5% in Iguhu, 23.6% in Emutete, 12.7% in Shikondi, 9.6% in Fort-Ternan and 10.6% in Marani. The mean malaria infection prevalence was 23.5% in Iguhu, 21.1% in Emutete, 5.1% in Shikondi, 3.1% in Fort-Ternan and 3.6% in Marani. There was a significant difference in the antibodies and malaria infection prevalence among the two valley systems and the plateau ($P<0.05$). There was no significant difference in the antibodies and malaria infection

prevalence within the U-shaped valleys and within the V-shaped valleys (P> 0.05). There was a 5.7 fold and a 2-fold greater parasites and antibody prevalence respectively, in the U-shaped compared to the V-shaped valleys. The plateau antibody and parasite prevalence was similar to that of the V-shaped valleys. U and V-shaped valleys have similar climate therefore the observed differences in parasites and antibody prevalence are likely to be due to their drainage characteristics.

76

SURVIVIN GENE EXPRESSION AND TOTAL P53 IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Treatment and prognosis are still problematic in management of acute leukemia patients. Survivin is one of the most cancer-specific proteins identified to date, being upregulated in almost all human tumors. Biologically, survivin has been shown to inhibit apoptosis, enhance proliferation and promote angiogenesis. The aim of this study is to detect the biologic and/or prognostic significance of survivin (S) expression and total P53 in acute lymphoblastic leukemia and its correlation to patients' outcome. Sixty two patients newly diagnosed acute lymphoblastic leukemia were followed up for 2 years or until death and they were treated with chemotherapy. Survivin protein and total human P53 were measured by quantitative sandwich enzyme immunoassay technique from peripheral blood from those patients at diagnosis and at complete remission. Twenty apparently healthy individuals were used as control group. A highly significant elevation in S protein and total P53 levels in acute lymphoblastic leukemia patients at diagnosis compared to controls. At complete remission no significant difference were found between acute leukemia patients at remission and healthy control group. S protein and total P53 was significantly higher in non-survived compared to survived group. A positive correlation was found between S gene expression level and total human P53 level in children with ALL. In conclusion, S protein related to anti-apoptotic proteins and act as the most widely characterized drug resistance mechanisms leading to unsuccessful treatment of ALL.

77

BEST PRACTICES AND INNOVATIONS WITH POTENTIAL TO INCREASE COVERAGE OF INTEGRATED COMMUNITY CASE MANAGEMENT OF COMMON CHILDHOOD ILLNESS - UGANDA AND MOZAMBIQUE

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During the last decade child mortality has reduced significantly in a number of African countries. Scale up of appropriate management of diarrhea, pneumonia and malaria was partly the reason behind the success. As a way of increasing access to treatment for sick children, several African countries are investing in community health workers (CHWs) to deliver integrated community case management (iCCM) for diarrhea, pneumonia and malaria. However, CHW programs have been faced with challenges of scale up while maintaining effectiveness, largely due to problems of ruptures in medicine supplies, lack of community involvement, shortfalls in training materials, lack of re-fresher training and supervision, high attrition and low performance of CHWs. The objective of this study was to identify best practices in starting up iCCM in Uganda and Mozambique and identify innovations with potential to increase coverage and improve its quality through better CHW performance and retention. A combination of methods will be used during the first 3 quarters of 2010, including consultative mapping, literature and policy document reviews, stakeholder interviews, focus group discussions and key informant interviews. Main outcomes will be an understanding of the key obstacles for regular and effective supervision; the contextual factors and ways which have an impact on CHW work motivation and satisfaction; the role of psychometric scales in measuring motivation; challenges and innovative solutions for information collection and flow; and improvements in delivery and content of iCCM training package. In conclusion, to reach the overall project goal of demonstrating that government led iCCM programs in 2 African countries can be driven to scale with quality solutions with potential to improve supervision, motivation and data use will be suggested. Innovations that have potential to address the project goal, but lack sufficient evidence of impact, will be formally evaluated through a randomized trial. The Ministries of Health will play major roles throughout the project by giving input into intervention design, participating in dissemination activities, involvement in development of implementation guidelines, supporting resource mobilization, supporting districts with regular medicine supply, and sustaining the program at national scale.

78

RENAL AND BLOOD PRESSURE LOWERING EFFECTS OF THE METHYLENE CHLORIDE-METHANOL EXTRACT OF THE STEM BARK OF *MAMMEA AFRICANA* SABINE (GUTTIFERAE)

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L-NAME induced hypertension has been associated with various renal morphological alterations. Previous studies have demonstrated that the methylene chloride-methanol (CH2Cl2-MeOH) stem bark extract of *Mammea africana* prevents the development of arterial hypertension in L-NAME treated rats. The purpose of the present work was to evaluate the effects of this extract on established hypertension and on renal function and morphology. Normotensive male Wistar rats were randomly assigned to control group (4% DMSO solution, per os for 4 or 6 weeks), L-NAME treated group (40 mg/kg/day, per os for 4 or 6 weeks), captopril group (L-NAME + captopril 20 mg/kg/day, per os) and *M. africana* group (L-NAME + M. africana 200mg/kg/day, per os). Captopril and the plant extract treatment were initiated 2 or 3 weeks after the beginning of L-NAME (40 mg/kg/day) treatment and were administered concomitantly with L-NAME for further 2 or 3 consecutive weeks. Systolic blood pressure (SBP) was recorded at baseline and at the end of every week by tail-cuff plethysmography. At the end of the experiment, urine, blood sample and kidneys were collected for creatinine clairance used as an estimation of glomerular filtration rate, plasma lipids determination and histological analysis. Captopril and M. africana significantly ($p < 0.001$) decreased blood pressure by 31.07 % and 30.59 % respectively in two weeks L-NAME hypertensive rats compared to animals receiving only L-NAME. But in 3 weeks L-NAME hypertensive rats both captopril and the plant extract failed to lower SBP. The administration of L-NAME for 6 weeks resulted in hyperlipidemia, a significant decrease ($p < 0.01$) in glomerular filtration rate, renal vascular thickness and lumen narrowing. These alterations were corrected by the plant extract demonstrating that *M. africana* is effective in managing moderate arterial hypertension and associated renal impairment. Thus it is a potential candidate for new antihypertensive drugs.

79

DENGUE HEMORRHAGIC FEVER: DIRECT COSTS AND CLINICAL FEATURES IN AN AMAZONIAN CITY IN NORTHERN PERU

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Despite the high prevalence of dengue virus (DENV) infection in the Amazonian region of Peru, incidence, clinical features, and economic consequences of dengue hemorrhagic fever (DHF) have not been well characterized. Herein, we describe these characteristics of the first documented cases of DHF identified from Hospital Santa Gema, in Yurimaguas, the second largest city in northeastern jungle region of Peru. Febrile patients were enrolled in our ongoing passive febrile surveillance study at which time an acute-phase blood sample was obtained. Patients with clinical alarm signs of DHF were hospitalized and monitored by medical staff and our research physician. Patient samples were processed for viral culture, RT-PCR, and ELISA IgM for recent DENV infection. A confirmed DHF case was a patient which presented with the four WHO criteria for DHF and had laboratory evidence of DENV infection. From 2005 to 2008, 1,024 febrile patients were enrolled, 37 (3.6%) of whom were suspected DHF cases. A total of 19 cases (51.4%) were confirmed as DENV infections, including 13 by virus isolation and six by IgM ELISA. Of the 13 patients confirmed by virus isolation, DENV-3 was recovered from 11. In addition there was one case of DENV-1 in 2005 and one case of DENV-4 in 2008. The principal alarming clinical sign was marked restlessness or lethargy (extreme prostration). Of the 19 confirmed DHF cases, 16 were grade II and 3 were grade III. All patients required hospitalization, one required intensive care in Lima, but no deaths were reported. The median age was 18 and 52.6% were male. The average duration of hospitalization was six days. The total direct cost for all suspected cases (bed days, laboratory tests, and radiologic tests) was $2,114.5 ($57.15 per person). The cost for confirmed cases was slightly higher ($62.30/case) than unconfirmed cases ($51.60/case), which should be put into the context of the local economy where a typical wage amounts to less than $50-60 per month. Additional costs and implications for the Health Sector will be discussed.

80

I TOLD YOU SO, WORDS OF WISDOM FROM YOUR WIFE CAN SAVE YOUR LIFE

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Fifty million people infected with dengue, the most prevalent mosquito borne viral disease each year. Most infections (90%) are asymptomatic. There are 4 related but distinct dengue viruses from the genus flavivirus. One serogroup does not offer cross protection to others. The second most common cause of fever in Western travelers to developing countries, symptoms develop 4-7 days after bite of Aedes egypti mosquito. Classic dengue fever includes headache, eye pain and severe myalgias- "breakbone fever". Exam is nonspecific, occasional macular rash. Leukopenia, thrombocytopenia and liver function abnormalities are seen. Diagnosis by serology. There is no treatment. Prevention is mosquito repellent, not bed netting, as mosquito bites during the day. Aerial spraying not effective, the mosquito breeds indoors. No vaccine is available, must contain all four serotypes. We present a case of two health care workers who returned to Trinidad with different outcomes. The wife used repellant but the husband did not. Unfortunately, he suffered the consequences. The subject was a 60 year old male complaining of severe headache, weakness and subjective fever, starting 4 days pta. Headache was L sided, constant, radiating to the neck and lower back, 8/10, no aggravating factors. Denies prior episode. No other associated symptoms. No significant medical history. Physical exam benign, except temp. of 38.5C and few petechiae on arms and legs. Initial platelet count 33 thousand only lab abnormality. On day 6 platelet count fell to 9 thousand after receiving 6 units of platelets. ALT and AST increased to 448 and 457 on day 9. Antibodies to dengue virus IgM and IgG positive at 1.2 and 7.6 (cutoff < 0.9). Patient given prednisone with improvement of platelet count.

The pathogenesis of thrombocytopenia in dengue includes: bone marrow suppression, platelet destruction, and molecular mimicry between viral protein and coagulation factors like plasminogen. Monoclonal antibodies directed to the virus bind to human fibrinogen, platelets and endothelial cells in mice. Risk factors for severe DHF include serotype 2 and "Asian" genotypes, as well as prior infection, younger age, well nourished and white. Platelet transfusion indicated in severe thrombocytopenia; anti-D immune globulin is still investigational. No adequate prophylaxis, mosquito repellant is mandatory. Our patient now heeds his wife's advice.

81

PRE-TRAVEL COUNSELING PROGRAM IN SAIPEM'S OCCUPATIONAL HEALTH DEPARTMENT FOR THE PREVENTION OF INFECTIOUS DISEASES

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Saipem, with more than 38,000 employees, is one of the biggest contractor company in oil and gas industry. The workplace is in ra emote area with a high risks of infectious diseases. The Occupational Health Department has developed a pre travel counseling program for the prevention of infectious risks. Pre travel Counseling is a fundamental step before that employee leave to worksite. He will stay for few days or for many years in a country where there are infectious risks. Employee receives information regarding the country risk through leaflets (Hepatitis A e B, Malaria, Typhoid fever, Sexual transmitted diseases, etc), health booklet for travelling workers, and all the protective means (vaccination, malaria prophylaxis). The Saipem Medical Department through qualified Occupational Health personnel, gives the employee in a properly manner, details information regarding infectious disease and the possibility to be submitted to a vaccination program before he leaves. All vaccinations are noted in his vaccination booklet and he has the possibility to receive the booster in our medical Unit in the country where he will work. In case of Malaria he will receive information for avoid mosquito bites and the correct manner to take the chemoprophylaxis with the correct drugs (Atovaquone + Proguanil, Mefloquine, Cloroquine). During his stay in the workplace, employee has the possibility to continue the counseling program. An example is our two major courses that are mandatory: sexual transmitted diseases and the malaria control program. Full implementation of prevention programs like counseling brings an added value to both the employee and the company. Statistics show a decrease of infectious diseases in our workforce since the counseling program was established and implemented.

82

EVALUATION OF THE EXTRACTS OF THE INDIAN MEDICINAL HERB *PHYSALIS MINIMA* L. FOR ANTIOXIDANT ACTIVITY AND INHIBITORY POTENTIAL AGAINST KEY ENZYMES RELEVANT TO ALZHEIMER'S DISEASE AND HYPERGLYCEMIA

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Physalis minima L. (Solanaceae) is well known in the Indian traditional medicinal system as a remedy for spleen disorder and as a tonic, diuretic and purgative. The plant is also extensively used for cancer, inflammation and diabetes. In the present investigation, the aerial part of *P. minima* was successively extracted with petroleum ether, chloroform, ethyl acetate, acetone and methanol. The extracts were investigated for *in vitro* antioxidant activities and inhibition of the key enzymes acetylcholinesterase involved in Alzheimer's disease and α- glucosidase and

α-amylase relevant to type 2 diabetes. The plant extracts had substantial concentration of total phenolics, tannins and flavonoids. In antioxidant activity assays, the acetone and methanol extracts showed the maximum reducing power and DPPH and ABTS + scavenging activities, which were highly correlated with the total phenolic contents (R2=0.9822, R2=0.8801 and R2=0.8840, respectively). In contrast, the low polar extracts such as chloroform and ethyl acetate exhibited higher levels of Fe2+ chelating ability. All the extracts were found to have a dose dependant activity in DPPH, superoxide, hydroxyl and nitric oxide scavenging, and lipid peroxidation inhibition assays. Further, the methanol and acetone extracts showed marked inhibition on the activities of acetycholinesterase and α- glucosidase whereas the ethyl acetate extract significantly inhibited the activity of α- amylase over other extracts. The results of the study will lead in favour of the use of this plant as a potential additive for the replacement of synthetic antioxidant compounds. Further, the inhibitory activity on acetylcholinesterase, α- glucosidase and α-amylase highlights its medicinal property. Isolation and characterization of the bioactive constituents from the active fractions are in progress in our laboratory.

83

WHAT WOULD PCR ASSESSMENT CHANGE IN THE MANAGEMENT OF FEVERS IN A MALARIA ENDEMIC AREA? A SCHOOL-BASED STUDY IN BENIN IN CHILDREN WITH AND WITHOUT FEVER

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We recently showed in a school-based study in Benin, that applying a policy of anti-malarial prescriptions restricted to parasitologically-confirmed cases on the management of fever is safe and feasible. Additional PCR data were analyzed in order to touch patho-physiological issues, such as the triggering of a malaria attack or the usefulness of PCR in the management of malaria in an endemic area. PCR data were prospectively collected in the setting of an exposed (with fever) / non exposed (without fever) study design. All children had a negative RDT at baseline, were followed up to day 14 and did not receive drugs with anti-malarial activity. The index group was defined by children with fever at baseline and the control group by children without fever at baseline. Children at high risk for malaria in these two groups were defined by a positive PCR at baseline. PCR was positive in 66 (27%) children of the index group and in 104 (44%) children of the control group respectively. The only significant factor positively related to PCR positivity at baseline was the clinical status (control group). When definition of malaria attacks included PCR results, no difference of malaria incidence was observed between the index and control groups, neither in the whole cohort, nor in children at high risk of malaria. The rate of undiagnosed malaria at baseline was estimated to 3.7% at baseline in the index group. In conclusion, non malarial fevers do not or do not frequently trigger malaria attacks in children at high risk for malaria. Treating all children with fever and a positive PCR would have led to a significant increase of antimalarial consumption, with few benefits in terms of clinical events.

84

DELAYED TREATMENT IN TYPHOID PATIENTS WITH PERFORATED BOWEL IN NIGERIA: WHAT ARE THE CAUSES AND EFFECTS?

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The early nonspecific features of typhoid often result in patients mistaking their illness for malaria or something less serious. This fact, along with a poorly regulated healthcare system and lack of patient knowledge concerning where and when to seek healthcare, may lead patients in Nigeria to take actions that delay appropriate treatment. It is unknown whether these actions and the ensuing delays, along with delays encountered post-presentation, impact mortality. The objectives of this study was to identify factors that cause treatment delay and determine their impact on mortality in typhoid patients with perforated bowel at Baptist Medical Centre in Ogbomoso, Nigeria (BMCO). We reviewed all charts of typhoid patients admitted to BMCO for surgical repair of perforated bowel from January 2004 to March 2009. There were 173 patients treated during that period; however, adequate records were obtained for 144 patients. These were analyzed for relationships between various treatments/factors and delayed presentation/mortality. Most patients (88%) received treatment before presenting to BMCO for surgical repair of perforated bowel. Patients received treatment from private clinics (67%) and traditional healers (8%) and also self administered pharmaceuticals (23%) and herbal remedies (5%). Eleven percent of patients reported having been treated for malaria. Associations between delayed presentation were found with receiving any pre-presentation treatment (p=0.005) and treatment at a private clinic (p=0.009). Treatment delays following presentation were due to difficulties paying the required surgical fee (19%) and obtaining blood for transfusion pre-operatively (11%) and post-operatively (5%). Having a delay in securing blood pre-operatively was associated with increased mortality (p=0.028). Increased mortality rates were also found for longer durations of that delay (p=0.037) and the presentation-surgery time interval (p=0.025). In conclusion, several factors delay treatment and impact mortality of typhoid patients with perforated bowel. Though financial hardship plays a prominent role in treatment delay, a multifaceted approach that includes education of patients and community healthcare providers; elimination of required surgical fees; and efforts to increase blood donation can ensure that patients with typhoid present for and receive proper treatment as quickly as possible.

85

CHEMOKINES AND CYTOKINES INDUCED BY MONOCYTES EXPRESSING DENGUE VIRUS NONSTRUCTURAL PROTEINS NS4B AND NS5 STIMULATE MICROVASCULAR ENDOTHELIAL CELL ADHESION MOLECULES

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Dengue virus (DENV) continues to spread worldwide and the incidence of dengue hemorrhagic fever (DHF) is on the rise. DHF immunopathology involves elevated levels of circulating chemokines and cytokines which stimulate the expression of adhesion molecules on vascular endothelial cells during acute infection. DENV has a plus-sense RNA genome encoding for three structural and seven nonstructural proteins (NS). Previous data demonstrated that NS5 can induce interleukin-8 (IL-8) but whether NS5 or other NS induce host immunomediators involved in endothelial cell activation remains unclear. We cloned each nonstructural gene of

the DENV type 2 New Guinea C (NGC) strain and either infected or transfected the monocytic cell line, THP-1, with the wild-type (wt) virus or DENV nonstructural plasmids, respectively. Further, we analyzed the culture supernatant for secreted immunomediators using the Luminex technology followed by incubation of UV-treated THP-1 culture supernatant with human microvascular endothelial cells (HMVEC). Changes in the expression of ICAM-1, VCAM-1 and E-selectin were measured using quantitative real-time RT-PCR (qRT-PCR) and Western blot. Our plaque assay, qRT-PCR and Luminex data demonstrated that maximum DENV titers and RNA copies in THP-1 infected or transfected cells correlated with significant secretion of IL-6, IL-8, IP-10, TNFα or INFγ. Subsequent incubation of UV-treated THP-1-infected culture supernatant with HMVEC significantly stimulated the expression of VCAM-1 and E-selectin, but not ICAM-1. Furthermore, when expressed in THP-1 cells, NS4B and NS5 induced IL-6, IL-8, IP-10 and IFNγ, which also stimulated HMVEC VCAM-1 and E-selectin production. In conclusion, we present here for the first time an *in-vitro* model consisting of a monocytic cell line that supports both wt-DENV infection and DENV NS expression as well as primary HMVEC that show variable modulation of adhesion molecules. Our results indicate that DENV NS4B and NS5 induce chemokines and cytokines that stimulate the expression of HMVEC adhesion molecules similar to that of wt-DENV infection. These findings provide insight into viral-host interactions and responses that may be exploited by therapeutic interventions to mitigate DHF.

86

INVESTIGATIONS OF DENGUE VIRUS ENTRY IN MEGAKARYOCYTE CELL LINES

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Dengue virus (DV) is endemic in virtually every country in the tropics and subtropics and causes frequent outbreaks, making it one of the most important vector-borne pathogens today. It is estimated that two-fifths of the world's population is at risk of infection and about 50 million dengue infections occur every year. Though dengue normally causes a self-limiting infection, some patients may develop a life-threatening illness, dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS). Viremia and thrombocytopenia are the dominant clinical features and are well-correlated with disease severity. Despite the amount of research conducted on dengue disease, the host cell(s) accounting for viremia and cell receptor(s) for viral entry remain elusive. Previous research utilizing the rhesus macaque model indicated that DV antigen can be found in megakaryocytes. To investigate their role in the dengue life cycle, we used CHRF-288-11, a megakaryoblastic cell line, which can be stimulated into megakaryocytes and produce platelets, and K562, a progenitor erythroleukemia cell line and close relative of megakaryoblasts. The kinetics of dengue viral RNA amplification within these cell lines were determined with DV2 by quantitative real-time RT-PCR. K562 is capable of supporting dengue virus replication, whereas CHRF-288-11 is less permissive for dengue virus replication. Our hypothesis is that dengue virus may enter progenitor cells and differentiate them into their preferred cell type. Future investigations will focus on the binding and entry into these cell lines using reporter DV VLPs (virus like particles). These will be constructed by transfecting a plasmid containing structural genes of dengue 16881 into K562 cells, which stably express EGFP-DV replicon. These EGFP-DV VLPs will allow us not only to distinguish which cells could be infected but also to dissect which receptor(s) are responsible for binding and entry.

87

DENGUE IN RURAL NORTHERN COASTAL ECUADOR

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Dengue is increasingly prevalent in rural areas, but the factors that drive transmission in these settings remain largely unknown. An unpublished pilot study completed in 2007 demonstrated high prevalence of dengue antibodies in the population living in remote villages in north coastal Ecuador, and confirmed that *Aedes aegypti* is present in these villages. In the last two years, the first two cases of DHF in the area were recorded at the local hospital, indicating that dengue is a serious concern in this region. Construction of a new highway may affect dengue transmission in this area through increased travel to coastal urban areas (known centers of dengue transmission), or through environmental change, creating a vector population sufficient to sustain local transmission. We examine the effect of this highway on dengue in eight communities with varying levels of road access, all part of a larger epidemiologic study. The objective of this project is to isolate and sequence dengue virus from mosquitoes and from whole blood and serum samples from suspected and laboratory confirmed clinical dengue cases in the area, providing data on circulating dengue strains. Viral presence in mosquitoes and homology to virues isolated from clinical specimens confirms local transmission in the villages. Conversely, low sequence homology or lack of mosquito infection supports the hypothesis that infections are acquired elsewhere, linking current incidence to human movement patterns. Sequence data are also compared to reported sequences circulating elsewhere in South America. Molecular data complement the migration, epidemiological, and serological investigations in this region, providing a clearer picture of the sources and risk factors of dengue infection. This information can be used to develop effective and cost-efficient interventions that can reduce disease associated morbidity and mortality and preserve the health of the people in this region.

88

INVESTIGATING DENGUE VIRUS SEROTYPE-SPECIFIC BIOMARKERS VIA MALDI-TOF/TOF

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Dengue is the most important viral vector-borne disease and more than 2.5 billion people are at risk for dengue infection, with 50 million infections occurring annually in over 100 tropical and sub-tropical countries. At least 500,000 people are hospitalized annually for dengue hemorrhagic fever (DHF), a more severe form of the disease, with fatality rates exceeding 20% in the absence of appropriate treatment. The onset of DHF occurs late in course of disease after the patient's fever subsides and the patient is sent home from the hospital and away from vital supportive therapies. Current diagnostic methods cannot predict which cases develop into DHF. Early identification of cases at risk for DHF at point of care (POC) could reduce mortality. Matrix assisted laser desorption/ionization time of flight time of flight (MALDI-TOF/TOF) is a technique which can be used to analyze the role of proteins in healthy and disease states and discover biomarkers which can be used to develop POC diagnostics. MALDI-TOF/TOF has been successfully used in the discovery of host biomarkers for cardiac disease and cancer and we have already used this technique to identify serum biomarkers which distinguish between DF and DHF. To aid development of a POC diagnostic test, we investigated dengue serotype-specific variation in biomarkers by screening serum from laboratory-confirmed cases of dengue virus serotypes 1-4 via MALDI-TOF/TOF. Preliminary results suggest no serotype-specific variation in DF biomarkers which may be important to incorporate in our previous studies

for severity of disease and aid the development of serotype specific DHF diagnostic tests. These results can be used to further develop diagnostic assays for point-of-care tests for clinicians.

89

VACCINATION AND HOMOTYPIC IMMUNITY RESTRAINS EMERGENCE POTENTIAL OF SYLVATIC DENGUE VIRUS TYPE 4 IN THE URBAN TRANSMISSION CYCLE

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Sylvatic dengue viruses (DENV) are both evolutionary and ecologically distinct from urban DENV. Sylvatic DENV are maintained in an enzootic transmission cycle most likely between non-human primates and arboreal *Aedes* spp. mosquitoes. Recent evidence from West Africa and Southeast Asia suggest that sylvatic DENV come into regular contact with humans, where *Ae. furcifer* and *Ae. albopictus* respectively, may act as bridge vectors between forest and peridomestic habitats. The ability of sylvatic DENV to emerge into an urban transmission cycle may limit the potential for eradicating dengue with vaccines currently in late stages of development. Here we assessed the likelihood of sylvatic DENV-4 emergence in the face of natural immunity to current endemic strains and to two vaccine candidates. Our data, based on the capacity of primary DENV-4 infection sera from convalescent patients and vaccinees to neutralize both endemic and sylvatic DENV-4 strains, indicate homotypic cross-immunity but limited heterotypic immunity. Therefore, emergence of sylvatic strains into an urban cycle would appear to be limited by homotypic immunity.

90

SEROPREVALENCE OF DENGUE FEVER IN U.S. ARMY SPECIAL OPERATIONS FORCES - INITIAL RESULTS AND THE WAY FORWARD

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Worldwide endemicity of dengue is increasing, making it a threat of particular concern to our United States Army Special Operations (USASOC) soldiers. These personnel routinely deploy to areas with multiple dengue serotypes, potentially increasing the risk of more severe disease manifestations and loss or degradation of operational capability. Since October 2008, at least nine USASOC soldiers have contracted dengue; four with multiple serotypes. We hypothesized that many more had been exposed. To characterize the risk, we initiated a seroprevalence study using approximately 500 samples from the DoD Serum Repository. The first stage of the study focused on personnel who served in the dengue endemic areas for at least 30 days from 2006 through 2008. An 11**%** seroprevalence rate, (determined using a sandwich ELISA procedure) confirmed our hypothesis, provides us with an epidemiologic baseline in our population, helps guide medical threat planning, and drives development of countermeasures while highlighting concerns about the introduction of dengue into non-endemic areas.

91

EVALUATION OF A RAPID ASSAY FOR DETECTION OF DENGUE EARLY MARKER NONSTRUCTURAL PROTEIN 1 (NS1)

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Dengue is a flavivirus found largely in areas of the tropics and sub-tropics, placing over half of the world's population at risk. Traditional methods for diagnosis of dengue include detection of serological markers such as IgM and IgG antibodies to dengue. Dengue NS1 is an early marker which can be detected in serum on day 1 after onset of fever and up to day 9. In comparison, an IgM response is not detectable until days 3 to 5. Rapid detection of dengue NS1 antigen is a valuable procedure, as it allows detection of infection prior to seroconversion. Early diagnosis of dengue allows promote implementation of supportive therapy and monitoring which reduces risk of severe complications such as Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS), especially in dengue endemic countries. The Panbio Dengue Early Rapid detects dengue NS1 in sera. The dipstick test was evaluated at two reference laboratories in South-East Asia. The studies used samples from acute fever patients (days 1 to 9 post onset of illness) characterised for dengue by a combination of PCR, virus culture, hemagglutination inhibition (HAI) and IgM and IgG ELISA. Positive samples were representative of primary or secondary infections with different serotypes. The results reported from the two sites were 71.1%, 68.9% sensitivity and 96.0%, 96.7% specificity respectively. Importantly, when combined with the Panbio Dengue Duo Cassette (IgM and IgG), the overall sensitivity increased to 93.5%. These results demonstrate that the Panbio Dengue Early Rapid is a valuable tool in the early diagnosis of dengue.

92

MOLECULAR EPIDEMIOLOGY OF DENGUE 2 VIRUS IN PERU

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Dengue (DEN) virus is a member of the family *Flaviviridae*, genus *Flavivirus*, and is responsible for more than 50-100 million cases annually throughout the world. Dengue infection is caused by four different dengue serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) and the clinical spectrum of disease ranges from dengue fever, dengue hemorrhagic fever (DHF), dengue shock syndrome, and death. Epidemiological information has linked the development of DHF with secondary dengue infections and has also suggested that certain DEN strains are more virulent than others. In 1995, the first cases of DEN-2 were detected in Iquitos and later spread to other areas in Peru; however, despite intensive surveillance efforts, no DHF cases were recognized. In 2001, the first DHF cases associated with DEN-2 virus were recognized in the coastal region of Peru. To investigate the genetic diversity of DEN-2 strains circulating in Peru before, during, and after the appearance of DHF cases, RNAs extracted from 58 viruses isolated in C6/36 (*Aedes albopictus*) cells were performed by RT-PCR. The envelope (E) and E/NS1 gene junction sequences were determined and used in a phylogenetic comparison with a global sample of DEN-2 viruses. Phylogenetic analysis revealed the circulation of two genotypes in Perú: American and American/Asian. Interestingly, the DEN-2 strains circulating prior to the reports of DHF only belong to the American genotypes whereas the DEN-2 strains isolated during and after 2000 belong primarily to the American/Asian genotype. Although clinical information is lacking for some of the cases included in the study, our results support previous findings of association of DHF with DEN-2 American/Asian genotype and highlight the need for continuous monitoring for the emergence of new DEN genotypes that may be associated with severe disease.

93

FOR WHAT IS A DENGUE VIRUS FIT?

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While phylogenetic analyses have demonstrated significant variation between the consensus sequences of dengue virus (DENV) populations, there is much less information about the, perhaps more relevant, variation within populations of DENV in natural hosts - humans and mosquitoes. The limited genetic data which is available for intra-DENV population variation has shown most genomes to contain changes/defects which would be expected to render the genome non-infectious. While these population studies also have identified recombinant DENV genomes, it is unclear whether these genomes are infectious or whether they are fit enough to enter natural cycles of transmission. The fitness of a DENV might be considered to have two major components, the ability to infect cells in an host and the yield of virus from infected cells. We have found that the C6-36 mosquito cell line is orders of magnitude more sensitive to infection with DENV 1 than human cell lines which are used commonly for the study of DENV (e.g. HuH7, HepG2, K562) such that some of the human cell lines could not be infected with these viruses. All cultures of C6-36 cells which could be infected with one infectious dose of DENV 1 released virions into the culture supernate which could be detected either in direct or virus-capture ELISAs. While all cultures achieved peak virus production by 8-10 days after infection, the amount of virus produced by each culture varied. Fewer than 10 percent of cultures infected with one infectious dose of DENV 1 produced more virus than cultures infected with undiluted (10^3 - 10^5 TCID) parental virus. This is interpreted as showing that most members of DENV 1 populations are less fit than the population as a whole. This could be interpreted as evidence for extensive complementation between members of DENV populations in host cells. It also suggests strategies that might be employed to enhance the effectiveness of the dengue vaccines currently under trial.

94

PAN-SEROTYPE SEQUENCE-SPECIFIC DETECTION OF DENGUE VIRUS USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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Dengue fever is one of the world's most common infectious diseases. Traditional laboratory diagnostic methods for dengue virus (DENV) such as virus isolation are labor-intensive and time-consuming. Polymerase Chain Reaction (PCR)-based diagnostic assays have proven to be very useful for accurate diagnosis of acute infectious diseases in a laboratory setting. Functioning similarly to PCR, loop-mediated isothermal amplification (LAMP) is a promising new technology that allows for rapid amplification of RNA or DNA. Unlike PCR, this technology does not require costly thermocyclers, and the results can be visualized using the naked eye. In this study, we investigated the feasibility of detecting all four serotypes of DENV utilizing a single set of degenerate primers. We successfully developed a reverse transcription (RT)-LAMP assay capable of detecting all serotypes of DENV in less than 60 minutes. We have also evaluated our assay using a panel of clinical samples obtained from either DENV-positive febrile patients or normal human sera and observed 86% sensitivity and 94% specificity. We have occasionally observed non-specific amplification in our assay, and have successfully implemented a novel technique designed to eliminate this problem. Using the re-designed assay, we have compared the limit of detection of our RT-LAMP to that of a popular DENV RT-PCR assay, using DEN3 as a model. Both assays were capable of detecting virus down to 10-100 copies/rxn. The ease and relative cost-effectiveness of this RT-LAMP assay makes it a promising candidate for point-of-care use, thereby reducing the time between symptom presentation and accurate diagnosis.

95

DENGUE VIRUS CROSS-PROTECTIVE IMMUNITY IN SHAPING HETEROGENEOUS SEROTYPE EPIDEMIC CIRCULATION PATTERNS, A STUDY OF DENGUE VIRUS TYPE 1 AND 4

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Mathematical modeling has postulated that the presence of moderate cross-protective antibodies from dengue virus type 4 (DENV-4) infected people may shape DENV-1 circulation resulting in alternating epidemic patterns of these two DENV serotypes circulating in Bangkok between 1973-2002. Previous phylogenetic analyses linked to the epidemic cycle also indicated that the DENV-1 clade extinction and replacement was the consequence of the DENV-4 cross-immunity selective pressure. In this study, we used the plaque reduction neutralization test to investigate if late convalescent (6 and 12 months) serum from 5 people infected with DENV-4 between 1994-1995 could cross neutralize 23 DENV-1 isolates representing both the modern existing (1990-2002) and the older extinct (1980-1994) DENV-1 clades. The results revealed that the antibody from known past DENV-4 infections exhibited varying levels of cross-reactivity against DENV-1 isolates compared with late convalescent DENV-1 infected control serum. Serum from one DENV-4 sample showed a marked increase in neutralization while the remaining 4 were reduced compared with the DENV-1 control serum. There was no measurable neutralization difference between the modern and extinct DENV-1 isolates. The small number DENV-4 infected people during this time frame and these results suggest that cross-immunity from DENV-4 infections have less effect on DENV-1 prevalence and/or its clade extinction and replacement. In addition, the replication rate of each serotype might be a factor effected to the epidemic pattern. Our examination on the replication rate of the wild type isolates from this period in *Aedes aegypti* mosquitoes showed a low DENV-4 replication rate in single, dual, and multiple infections.

96

UPDATE ON THE PHASE III STAGE SANOFI PASTEUR RECOMBINANT TETRAVALENT DENGUE VACCINE

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The development of the Dengue tetravalent (TV) recombinant YF17D-based chimeric vaccine has been continuously driven by a benefit/risk evaluation and mitigation strategy. Earlier pre-clinical studies have demonstrated that the sanofi pasteur dengue vaccine is genetically and phenotypically stable, non-hepatotropic, less neurovirulent than YF17D and does not infect mosquitoes by the oral route. *In vitro* and *in vivo* preclinical studies also show that the TV dengue vaccine induces controlled stimulation in human dendritic cells, and has significant immunogenicity in monkeys. Results of Phase II trials in the USA, the Philippines and Mexico show that the majority of adverse events are mild to moderate and transient in nature. Observed Viraemia is transient and low, and does not increase after the initial dengue TV administration, even in the case of incomplete responses. PRNT50 seroconversion ranges between 80 and 100% for all four serotypes in subjects injected with 2 to 3 doses of TV dengue vaccine. Responses have been monitored at the cellular level in humans: Th1 and CD8 responses are induced with an IFN-γ/TNF-α ratio favoring IFN-γ. A worldwide clinical development program for dengue TV is underway including completion of the enrollment of 4000 school-age children (4-11 years) in an efficacy trial in Thailand and the planned evaluation of industrial scale clinical lots in Phase III by year end. Assuming continued successful outcomes, initial submission to national regulatory authorities can now be considered within a 5-year period.

97

HIGH INCIDENCE OF PERIPHERAL BLOOD PLASMACYTOSIS IN PATIENTS WITH DENGUE VIRUS INFECTION: A PROSPECTIVE STUDY

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One of the characteristic features of dengue virus (DENV) infection is the occurrence of leucopenia and thrombocytopenia, probably resulting from virus induced bone marrow suppression. Despite the general bone marrow suppression, polyclonal peripheral blood plasmacytosis has occasionally been described in DENV infected patients. The frequency of blood plasmacytosis in patients with dengue infection, the origin of these plasma cells (PCs) and the mechanisms by which they appear in the blood are not known. We initiated this prospective observational study to quantify and describe the kinetics and phenotype of peripheral blood plasma cells (PCs) in these patients. Morphological examination and flow cytometric (FC) analysis for the characterization and immunophenotyping of lymphocyte subsets and PCs were performed in 35 and 31 patients suspected of DENV infection, respectively. Our results show that blood plasmacytosis is a very common hematological finding. Depending on the days of illness at presentation, blood plasmacytosis was observed in 64% to 73% of patients. Blood plasmacytosis was the most pronounced before 7 days of illness and declined rapidly thereafter, to completely disappear after 14 days of illness. Blood plasmacytosis was higher in secondary DENV infection. The majority of $CD138^+$ PCs (89%) had a shared immunophenotype ($CD45^+/CD19^-/CD56^-$) and in all cases the PCs were polyclonal. In conclusion, blood plasmacytosis is an unusual hematological finding that is most commonly seen in plasma cell leukemia or advanced stage multiple myeloma. However, blood plasmacytosis, characterized by a transient presence of polyclonal PCs in the circulation, is a common event in DENV infection. Blood PCs may play a role in the humoral immune response to and pathogenesis of dengue.

98

DENGUE DYNAMICS IN BINH THUAN PROVINCE, SOUTHERN VIETNAM: PERIODICITY, SYNCHRONICITY AND CLIMATE VARIABILITY

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Dengue is a major global public health problem with increasing incidence and geographic spread. The epidemiology is complex with long inter-epidemic intervals and endemic with seasonal fluctuations. This study was initiated to investigate dengue transmission dynamics in Binh Thuan province, southern Vietnam. Wavelet analyses were performed on time series of monthly notified dengue cases from January 1994 to June 2009 (i) to detect and quantify dengue periodicity, (ii) to describe synchrony patterns in both time and space, (iii) to investigate the spatio-temporal waves and (iv) to associate the relationship between dengue incidence and El Niño-Southern Oscillation (ENSO) indices in Binh Thuan province, southern Vietnam. We demonstrate a continuous annual mode of oscillation and a multi-annual cycle of around 2-3-years was solely observed from 1996-2001. Synchrony in time and between districts was detected for both the annual and 2-3-year cycle. Phase differences used to describe the spatio-temporal patterns suggested that the seasonal wave of infection was either synchronous among all districts or moving away from Phan Thiet district. The 2-3-year periodic wave was moving towards, rather than away from Phan Thiet district. A strong non-stationary association between ENSO indices and climate variables with dengue incidence in the 2-3-year periodic band was found. In conclusion, multi-annual mode of oscillation was observed and these 2-3-year waves of infection probably started outside Binh Thuan province. Associations with climatic variables were observed with dengue incidence. Here, we have provided insight in dengue population transmission dynamics over the past 14.5 years. Further studies on an extensive time series dataset are needed to test the hypothesis that epidemics emanate from larger cities in southern Vietnam.

99

DEFINING DETERMINANTS OF ANTIBODY PROTECTION AND ENHANCEMENT IN A NOVEL MOUSE MODEL OF DENGUE VIRUS INFECTION AND DISEASE

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The development of a mouse model to study dengue virus (DENV) infection and disease has provided the opportunity to pursue investigations of antibody determinants of protection and enhancement. Using an AG129 mouse model that reproduces lethal antibody-dependent enhancement (ADE) as well as antibody-mediated protection, we first showed that the interaction of the Fc portion of the antibody-virus complex with Fcγ receptors on target cells is required for ADE *in vitro* and *in vivo*. Now we are dissecting the role of DENV antibodies at the envelope (E) protein domain- and epitope-specific levels using both human and mouse sera and monoclonal Abs (MAbs). To address the contribution of specific antibodies to protection and enhancement of DENV infection, we have depleted murine anti-DENV serum of Domain (D) III-specific Abs. *In vitro* analysis indicates a 75% reduction in functional neutralization capacity upon EDIII-antibody depletion. The effect of transferring to mice intact, control depleted, and EDIII-depleted sera adjusted to equal neutralizing titer followed by DENV challenge will be reported. To further dissect the role of E-specific antibodies, MAbs of different epitope specificities and neutralization capacity were selected to identify the relative importance of each component in modulating disease enhancement or protection. Initial characterization of both murine and human MAbs indicates that MAbs with higher neutralization titer are more effective at neutralizing a sub-lethal DENV infection and less efficient at enhancement than less neutralizing MAbs with comparable epitope specificity, thus indicating an important role for neutralization titer. We have also shown that genetically engineered mouse and human MAbs incapable of interacting with the Fcγ receptor can be therapeutic in mice and prevent ADE-mediated disease when administered after lethal challenge. Analysis of different types of MAbs in this assay has revealed that modified serotype-cross-reactive mAbs directed to the EDII fusion loop are protective ($p<0.05$) and significantly reduce viral load, and that therapeutic neutralizing potential may be predicated upon epitope specificity. These studies are elucidating information about how specific E protein domains and epitopes contribute to protection or enhancement of dengue disease that should be useful for development of safe and effective dengue vaccines and antibody-based therapeutics.

100

UNUSUAL DENGUE VIRUS 3 EPIDEMIC IN NICARAGUA, 2009-2010

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Dengue has increased in incidence and severity in the Americas over the past 30 years. In 2009-10, Nicaragua experienced the largest dengue epidemic in over a decade, marked by unusual clinical presentation, as observed in two prospective studies of pediatric dengue in Managua. Between August 2009 and January 2010, 220 dengue cases were confirmed among 396 study participants at the National Pediatric Reference Hospital; overall, 5-10 times more suspected dengue was seen there than in prior years. In our pediatric cohort study, an incidence of 4.4% was observed (168 dengue cases) in the 2009-10 dengue season, compared to 0.4-1.9% in 2004-8 (13-65 cases). DENV-3, the predominant serotype in Managua since 2008, caused 89.4% (hospital) and 83.2% (cohort) of cases. In the hospital study, 24% of subjects were transferred to intensive care compared to 5-11% in 2005-8. Although fewer hospital cases in 2009-10 were classified as DHF/DSS (13.6% vs 21-61% in 2005-8), "compensated shock" (≥2 of: cold extremities, poor capillary refill, tachycardia, tachypnea, rapid pulse) was observed in more cases (37.3% vs 9-14.6%). Signs of poor perfusion presented 1-2 days earlier in 2009-10 than 2005-8, but generally did not progress to hypovolemic shock, possibly due to early IV fluid therapy. In Kaplan-Meier survival analysis, "compensated shock", slow capillary refill, and cold extremities presented significantly earlier in 2009 after adjusting for day of presentation to the hospital ($p<0.005$). Similar results were obtained in the cohort. Several hypotheses are now being tested to explain the high incidence and distinct clinical presentation of dengue in 2009-10. Preliminary results of full-length sequencing of DENV-3 do not reveal a major shift in clade between 2008 and 2009, though further sequencing is underway. Due to a pandemic influenza A H1N1 epidemic just before the dengue epidemic, levels of antibodies to H1N1pdm antigen in cohort dengue cases are being tested to determine whether recent influenza infection modulated subsequent dengue cases. The effects of case management and prior DENV infection on disease progression are also under analysis. Finally, DENV antibodies in paired annual blood samples from cohort subjects will be analyzed to determine the incidence of overall DENV transmission in 2009-10 compared to previous years. These studies should improve our understanding of determinants of the varied disease manifestations of dengue.

101

RESULTS OF BASELINE ASSESSMENTS OF COMMUNITY CASE MANAGEMENT SUPPLY CHAINS IN MALAWI AND ETHIOPIA

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Slow progress in effectively treating childhood diseases has influenced governments, NGOs, and donors to adopt new approaches to delivering life-saving health services for young children. Community case management (CCM) is one promising approach which aims to reach children and reduce childhood mortality in underdeveloped areas by training community health workers (CHWs) to treat common childhood illnesses at the community level. Evidence suggests, however, that inconsistent supplies pose important constraints to CCM programs, often adversely affecting outcomes. The Improving Supply Chains for Community Case Management (SC4CCM) Project aims to demonstrate that supply chain constraints at the community level can be overcome, potentially yielding significant improvements in CCM effectiveness and impact. SC4CCM will introduce innovative approaches to strengthen supply chains and effectively deliver key drugs to CHWs, but there is little knowledge in most countries regarding current supply chain practices at the community level. In Malawi and Ethiopia, the first two SC4CCM focus countries, baseline assessments will be carried out during early 2010 to determine current conditions and provide evidence for effective interventions. Methodology will include physical counts of CCM products at CHWs and their resupply points, and structured interviews with CHWs and other stock managers. Experimental and control districts will be selected purposefully, with health facilities sampled randomly within selected districts, and 2-3 CHWs selected randomly per selected facility. The presentation will describe baseline findings, focusing on supply chain strengths and weaknesses, how findings inform program planning and innovations, how interventions will be tested, and potential applications for other settings.

102

USING A THEORY OF CHANGE MODEL TO IMPROVE SUPPLY CHAINS FOR COMMUNITY CASE MANAGEMENT IN RESOURCE LIMITED SETTINGS

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The Improving Supply Chains for Community Case Management of Pneumonia and Other Common Diseases of Childhood (SC4CCM) project has designed a theory of change (TOC) model that clearly maps out the project's goals and objectives and the steps it will take to achieve these. Community case management (CCM) is an approach designed to reduce childhood mortality by reaching more children in resource limited settings, however evidence shows that CCM is hampered by inconsistent availability of appropriate, quality and affordable medicines. The SC4CCM project aims to demonstrate in four sub-Saharan African countries that supply chain constraints at the community level can be overcome. The SC4CCM TOC model identifies the distinct building blocks that, in combination, comprise an effective supply chain for reaching community health workers in remote and isolated areas. It links interventions, assumptions and indicators to final outcomes and provides a roadmap for measuring and institutionalizing change. While the building blocks are relevant for all supply chains for CCM commodities, the individual interventions and targets for each indicator will be country-specific. The TOC framework will be used to develop country specific implementation and evaluation plans. The SC4CCM TOC will provide an iterative framework that will guide progress while being continuously refined over the course of the project. The final outcome will be a TOC model that can be scaled up and applied to other CCM projects or programs to improve availability of the appropriate quality, affordable commodities at the community level and other levels of the health system.

103

LEAD IS UNIFORMLY DETECTED IN A SAMPLE OF CHILDREN ATTENDING OUTPATIENT PEDIATRIC CARE IN ASMARA

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The objective of this study was to determine the prevalence of blood lead levels in Eritrean children. Cross sectional study of 120 children aged 12-71 months oldstaken from a convenience sample of non-seriously ill children presenting to the Orotta Children's Hospital outpatient department during August 2009. After obtaining informed consent from a parent, whole blood was collected from a peripheral vein for blood lead level (BLL) and complete blood count (CBC) determination. The BLL was

performed at the Mayo Clinic sponsored by Washington University in St. Louis, Missouri, while the CBC analysis was performed at the National Health Laboratory of Eritrea in Asmara, Eritrea. Lead was detected in all blood specimens. Mean BLL was 5.0 + 2.9 mcg/dL, median 4.0 mcg/dL, with a range of 0.5 to 16 mcg/dL. There was a significant and direct relationship between BLL and hemoglobin level, with higher levels of hemoglobin found at higher levels of BLL (95% CI 0.01 to 0.62; p=0.043). Conversely there was a significant negative relationship between erythrocyte mean corpuscular volume (MCV) and BLL (CI -0.12 to -0.002 p=0.042), with higher BLL associated with lower MCV. In conclusion, lead was uniformly detected in blood of Eritrean children aged 1 to 5 years old. Further study of environmental causes for BLL is warranted. These findings suggest the need to make the public, government organizations, and health professionals aware of the risk factors for and prevention strategies to mitigate lead exposure.

104

REASONS FOR BREAST FEEDING TERMINATION IN A POOR PERIURBAN COMMUNITY IN THE DOMINICAN REPUBLIC

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Longer duration of breastfeeding plays an important role in improving child health and reducing mortality. However, breastfeeding length is often suboptimal. Further information is needed on factors impeding longer duration of breastfeeding to inform health promotion efforts aimed at improving the length of breastfeeding. The aim of this study was to determine reasons for breastfeeding termination of mothers in a poor periurban community in the Dominican Republic using a mixed questioning approach. A structured questionnaire was administered through an interview with all mothers or alternative caregivers of children under the age of five who participated in a growth-monitoring program in a Haitian Batey in the Dominican Republic. Reasons for breastfeeding termination were obtained through a combination of an open-ended question and a series of close-ended questions, which had been formulated from previous research on breastfeeding in the Dominican Republic. Of 132 participants, 79 (60%) had terminated breastfeeding prior to the interview. Of this subgroup, 19% terminated breastfeeding at less than six months and 65% terminated breastfeeding at less than two years. A new pregnancy (22%) was the most common reason for breast feeding termination in response to the open-ended question. Of the ten closed-ended questions, "it was time" to stop breastfeeding was the most frequently endorsed (46%). Of those who terminated at less than six months, the most commonly endorsed reason was that the child did not want the breast (53%). In conclusion, breastfeeding duration in this community is suboptimal. Use of different styles of questions identified different sets of breastfeeding termination reasons, which may better inform health promotion efforts aimed at increasing breastfeeding duration.

105

INHERITED BLOOD DISORDERS: AN ONLINE DATABASE OF ALLELE FREQUENCIES TO REFINE HEALTH METRICS

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A good knowledge of the spatial distribution of diseases is crucial for guiding efficient public health policy. Inherited blood disorders include both the commonest monogenic diseases (haemoglobin structural variants and thalassaemias) and the most common human enzyme defect (G6PD deficiency) worldwide. Their impact on public health is growing both in developed countries due to human migrations and in developing countries due to epidemiological transitions. Nevertheless, our knowledge about their current distribution and burden remains very poor, even in developed countries like the U.S. In order to improve the current situation, inherited blood disorders have recently been included in the Global Burden of Disease Program. The public health significance of sickle cell disease was also acknowledged last year, with the United Nations declaring a World Sickle Cell Day. Using a similar approach to the one used for *Plasmodium falciparum* and *P. vivax*, the Malaria Atlas Project has started creating a global open-access comprehensive database of inherited blood disorder allele frequencies. This database focuses on the most prevalent disorders selected for their malaria protective mechanism: haemoglobin S (HbS), haemoglobin C (HbC), glucose-6-phosphate dehydrogenase (G6PD) deficiency, South-East Asian ovalocytosis and Duffy negativity. The database will also ultimately include the thalassaemias and haemoglobin E (HbE). In an effort to help improve health metrics, especially in the developing world, this database, combined with model-based geostatistics and high resolution population data, allows the development of global contemporary maps for each of these disorders, and the refining of burden estimates. The development of such a resource highlights the difficulties in accessing some of the existing data and also flags up those areas from which data are really lacking.

106

SOCIOECONOMIC INEQUALITY IN UNDER FIVE MORTALITY: EVIDENCE FROM NAVRONGO DSS

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Improving the health of the poor and reducing health inequalities between the poor and non-poor has become central goals of certain international organizations like World Bank and WHO, including national governments in the contexts of their domestic policies and development assistance programmes.

There are also unquantified and poorly understood inequalities in access to health services within and between various population groups. Little is known about the factors that determine these inequalities and the mechanisms through which they operate in various sub-groups. The aim of the study was to first to describe under-five mortality trend according to wealth index, second to describe risk factors for under five mortality and finally to investigate the relationship between socio-economic and demographic factors and under five mortality during the period 2001 2006. The study involved all children born in 2001-2006. A total of 22,422 children younger than 5 years were found in 21,494 households yielding to 49275.13 Person-Years Observed (PYOs) up to 31st December 2006. Household wealth index was constructed by use of Principal Component Analysis (PCA), as a proxy measure of each household SES. From this index households were categorized into five quintiles (i.e., poorest, poorer, poor, less poor and least poor). Life table estimates were used to estimate mortality rates per 1000 PYO for infants (0-1), childhood (1-5) and under-fives children. Health inequality was measured by poorest to least poor mortality rate ratio and by computing mortality concentration indices. Trend test chi-square was used to determine significance in gradient of mortality rates across wealth index quintiles. Risk factors of child mortality were assessed by the use of Cox proportional hazard regression taking into account potential confounders. The results indicates unexpected low mortality rate for infant (33.4 per 1,000 PYO, 95% CI (30.4 - 35.6)) and childhood (34.7 per 1,000 PYO, 95% CI (29.9 - 40.3)). Under-five mortality rate was 90.7 per 1,000 PYO (95% CI (75.6 - 108.0)). The poorest to least poor ratio were 1.1, 1.9 and 1.5 for infants, childhood, and under-five year olds respectively, indicating that children in the poorest quintile were more likely to die as compared to those in the least poor household. Computed values for concentration indices were negative (infant C= -0.02, children C= -0.09 and under-five C= -0.04) indicating a disproportionate concentration of under-five mortality among the poor. The mortality rates trend test chi-square across wealth index quintiles were significant for both childhood (P=0.004) and under-five year old children

(P<0.005) but not for infants (P=0.134). In univariate Cox proportional hazard regression, children in the least poor households were shown to have a 35% reduced risks of dying as compared to children in the poorest category [crude H.R =0.65, P=0.001, 95% C.I (0.50 - 0.84)]. The results shown that for under five children, a boy is 1.15 times more likely to die as compared to a girl [crude H.R =1.14, P=0.038, 95% C.I (1.00 - 1.31)]. Second born had a 18% reduced risk of dying as compared to first born [crude H.R =0.82, P=0.048, 95% C.I (0.67 - 0.99)]. After controlling for potential confounders, the adjusted hazard ratio for wealth index decreased slightly. The estimated hazard for wealth index in the univariate was 0.65 while in the multivariate modeling the estimated hazard ratio is 0.60 in the first model. In conclusion, the study shows that household socio-economic inequality is associated with under-five mortality in Navrongo DSS. The findings suggest that reductions in infant, childhood, and under five mortalities are mainly conditional in health and education interventions as well as socioeconomic position of households. The findings further call for more pragmatic strategies or approaches for reducing health inequalities. These could include reforms in the health sector to provide more equitable resource allocation. Improvement in the quality of the health services offered to the poor and redesigning interventions and their delivery to ensure they are more inclined to the poor.

107

COMMUNITY PERMISSION AND INDIVIDUAL CONSENT PROCESS IN A LOW LITERACY SETTING

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Clinical trials have begun to be conducted in Mali only recently. In keeping with local traditions and with ethical requirements, community permission and then individual consent are necessary. Given the low literacy rate in Mali, it is important to adapt the methods of obtaining consent so that all participants may understand. As an attempt to ensure an accurate understanding of the necessary information, our investigators have the consent form translated into the local language, Bambara, and recorded on audiotape by the Malian National Center for Resources for Non-formal Education. This recording is then used during the meetings to obtain community permission as well as individual consent. Potential participants are encouraged to ask any questions they may have. Finally, community permission and individual consent are documented on a printed form in the official language, French. From 2006 to 2009, we completed 2 clinical trials of a novel conjugate vaccine and included 600 study participants. In total, 10 community meetings were attended by 359 community members and a total of 56 questions were asked. Questions concerned the risks, benefits, compensation and study design. Over the course of the studies, 33 persons withdrew consent; 28 did not provide a reason and 5 cited the blood draws. In conclusion, audiotaped translation of the consent form has been a useful tool during the informed consent process. The success of a study depends on the degree of understanding by the community. In the future, we plan to more formally evaluate the role of the audiotaped consent form.

108

DUST-OFF: PREDICTORS OF AMERICAN AIRAMBULANCE LOSSES DURING THE VIETNAM WAR

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The United States and its SEATO allies were involved respectively from 1960 - 1975 and 1962-1972. By the end of these periods, the United States committed approximately X number of troops and lost approximately 58,740. The primary SEATO ally, Australia, committed almost 500,000 troops, of whom almost 500 were lost. An important technological innovation, the helicopter, played a significant role in the mortality rate which the troops supplied. The Bell UH-IH, broadly known as the "Huey," became the "Dust off" for the medical evacuation of troops wounded in the field. Indeed, "dust off" informally entered the vernacular as the need - or act of- evacuation of personnel from an L-Z [Landing Zone] often designated as "hot," which meant that enemy troops were in close proximity. The symbol of the International Red Cross, worn by non-combatant medical personnel - not to mention emblazoned on the Huey its self - theoretically protected medics and their assistant as much as possible. The rules of war, however, seemed seldom applied to medics, Corpsmen, enew, and pilots who flew into the "hot" landing zones. As this paper will suggest, despite extraordinary measures to identify as clearly as possible the non-combat "Medevac Hueys" from the more ominous Hueys, equipped with a door gunner, firming an M-60 automatic machine gun. As Vietlong consistently refused to respect the Red Cross, American Medevac defended their aerocraft on both doors with MO Machines and in addition to the Red Cross Painted their Hueys white rather than green. Ironically, as the data suggest, specific factors - not least the bright white Hueys - because predictor of loss of crew members, patients, if not the helicopter itself.

109

THE IMPACT OF GLOBAL MIGRATION OF PEOPLE ON THE MORBIDITY OF CHRONIC PARASITIC INFECTIOUS DISEASES IN JAPAN

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As globalization proceeds, people migrate in a borderless manner worldwide. Japan is not exceptional, since much more foreigners have stayed here as residents who have originally come from countries, where chronic parasitic diseases are endemic. Some of the diseases were already eradicated from or have never existed in Japan. As there is little information regarding how many chronically-infected people reside in Japan, there is an urgent need to establish an appropriate screening system to detect such individuals to prevent unexpected accidents to occur. In this study, we conducted field surveillance to clarify the epidemiological status quo regarding how many foreign residents in Japan are actually being infected with chronic parasitic infectious diseases. We conducted medical examinations for foreign residents at 5 cities (Yamato, Fujisawa, Hiratsuka in Kanagawa, Ota in Gunma and Joso in Ibaraki) between 2007 and 2009. The medical check-up includes clinical interviews, blood tests for the detection of antibodies against parasitic diseases, stool examinations and ultrasonography. Among 473 people who accepted to provide with blood samples, 41 were determined as positive for antibodies (positive rate: 8.7 %) against parasites; 2 for malaria, 2 for visceral leishmaniasis, 1 for Chagas' disease, 19 for toxocariasis, 10 for gnathostomiasis, 5 for amoebiasis, 1 for trichinosis and 3 for schistosomiasis. 1 for echinococcosis. Stool examinations detected 16 positive cases among 263 people (positive rate: 6.1 %) who had provided with stool specimens. The positive cases include amoebiasis, giardiasis, cryptosporidiasis and Hookworm infection. As expected, there were no overt clinical symptoms among examinees. We conclude that the chronic parasitic infectious diseases are quite common among the foreign

residents in Japan when compared to the morbidity among indigenous Japanese people. Considering the well-established sanitary infrastructures in the Japanese Society, the immigration of chronically-infected people will not trigger the outbreak of parasitic diseases in Japan; however, transfusion-related infectious diseases such as Chagas' disease will have to be carefully monitored due to the lack of systematic screening system for the diseases in Japan.

110

HOW, WHERE AND WHY DO WE EVACUATE THOSE INFECTED WITH VIRAL HEMORRHAGIC FEVERS?

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The globalization of commerce is manifested by the migration of the workforce to third world environments. Such migration, particularly to the African continent, increases exposure to the traditionally neglected tropical diseases; viral hemorrhagic fevers. Although local, regional and national Departments of Health have attempted to implement educational and protective protocols, the ultimate treatment facilities for these lethal, highly contagious diseases, are usually not locally available. Beyond universal precautions transport guidelines/protocols are non-existent. Consequently a dismal prognosis is the rule. Our experience, in global medical assistance, reveals that the medical evacuation/transport of patients with Ebola, Lassa fever, Marburg etc. requires complete cooperation, as well as authorization, by all government agencies responsible for Public Health in the country of origin as well as the destination country. To address the transport of this unique and growing population of infected patients we have developed and used innovative safety measures to protect our medical teams/flight crews from contamination during medical evacuation/transport. Those measures include the design, in accordance with International Health Authority Guidelines (WHO, CDC), and implementation, of a compact, portable isolation unit (PIU), ideal for regional ground/air travel. More recently, we have incorporated a disposable biological containment unit (BCU) into our comprehensive protocols which is designed for a Gulfstream III, ideal for trans-ocean/continental travel. Both the PIU and BCU enhance our ability to medically transport infected patients. We have demonstrated that: (1) efficient movement of the sick/infected patients has a positive impact on their outcome and (2) the creation and credentialing of a global network of preferred providers willing and able to accept such patients facilitates the transfer to the nearest center of medical excellence rather than repatriations which may not always be practical.

111

THE USE OF SUPPORTIVE SUPERVISION TO STRENGTHEN IMMUNIZATION IN MALI

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In collaboration between the Malian Ministry of Health and the University of Maryland School of Medicine, training focusing on introduction of a new vaccine against *Hemophilus influenzae* type b was conducted. At training completion, evaluation showed that immunization information was not passed efficiently from district to health center level. To address this we employed a supportive supervision approach. Four supervisory tools, each focused on a specific category of immunization (injection safety, techniques and communications for immunization, program management, and surveillance) taking approximately 90 minutes to administer, were used. These supervisions were done at three month intervals for a year, with district level staff as part of the team. Before the year-long intervention started, evaluations were done in the four health centers in two regions where the interventions took place, and in four health centers in the same regions but in different districts, which served as controls. These evaluations covered injection safety, surveillance and program management, but did not completely correspond to the areas covered in the supervisory tools. The results of the evaluations showed that any intervention, even just performing an evaluation, had a positive outcome. For both the injection safety and surveillance evaluations, all eight centers improved performance, although those which had the supervisory visits were better performing overall in both regions. For vaccine management, all but one center in each region improved performance. In all centers where the quarterly intervention was done, we found an increased enthusiasm among staff to improve their performance and to ask questions about procedures as the year went on. The study will continue for another year with a crossover design, using the original tools in the control districts and four new tools in the original intervention areas.

112

METEOROLOGICAL SUPPORT THE PUBLIC HEALTH COMMUNITY

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There is a nexus point between public health, tropical medicine, and the environment. Reaching this point, however, requires strategic planning, trans-discipline partnerships, and resources to support new hybrid sciences (e.g., biometeorology). The American Meteorological Society (AMS) is the oldest professional society for the hydromet/climate/ocean sector in the U.S. and recognizes the importance that quality environmental data could bear in bolstering preparedness, improving surveillance, and expediting response in the public health community on monthly to seasonal timescales. We highlight that the impacts from a climate in transition make the union between health, medical, and environmental sciences even more pressing. Because of this, the AMS would like to report to the ASTMH community on our activities to inform our researchers and operational meteorologists, in the U.S. and overseas, about reaching out to your community. In addition, we would also like to hear from ASTMH members on how we could improve the delivery and quality of environmental data and strengthen partnerships in order to support your research and medical care regarding current and emerging diseases.

113

POVERTY, DIARRHEA, AND TREATMENT COSTS: UNRAVELING THE RELATIONSHIP

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In Bolivia, the under-five mortality rate is 65 per 1,000 children, and diarrhea is responsible for 37% of these deaths. Pediatric diarrhea causes a substantial economic burden to households and healthcare systems. The goal of this study was to identify demographic, etiologic, and behavioral factors associated with catastrophic costs resulting from treatment of gastroenteritis. We interviewed 384 caregivers of children with diarrhea from hospitals and outpatient clinics in 7 facilities across 4 Bolivian cities. Multivariate linear regression was used to identify predictors of increased treatment cost. A logistic regression model predicting the probability that a household's expenditure for treatment exceeded 1% of annual income (catastrophic cost) was constructed to identify determinants of catastrophic costs. Caregiver feelings about treatment costs and related coping mechanisms were also assessed. We identified demographic characteristics, treatment seeking behavior, and disease severity to be significant predictors of increasing cost burden and significant determinants catastrophic cost. Demographic characteristics, including male gender of child and city where treatment was sought, were correlated with increased treatment expenditures, suggesting a social bias in the seeking treatment for male children and overall higher treatment

costs in the southern lowlands. Treatment seeking behavior (increases in the number of places sought for treatment) predicted a significant increase in the cost burden. The majority of caregivers indicated that they had to find extra money to pay for treatment. More than 20% said that they had withheld treatment for their child because of high treatment costs. Coping mechanisms for high costs were significantly different for families who spent more than 1% of their income on treatment. Despite universal health insurance in Bolivia, pervasive use of health services contributes to caregiver costs. Effectively removing real and perceived barriers to healthcare are needed to achieve further reductions in diarrheal mortality in Bolivia.

114

A COMPARISON BETWEEN CAREGIVER KNOWLEDGE OF SIGNS OF DEHYDRATION AND PRIORITIZED HEALTH EDUCATION RECOMMENDATIONS IN PERI-URBAN DOMINICAN REPUBLIC

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Dehydration during diarrhoeal episodes is an important contributor to child mortality in the global south. Caregiver recognition of signs of dehydration may be a critical factor in prompting health restoring behaviours. Understanding existing caregiver knowledge relative to signs prioritized by health education recommendations may inform refinement of health education campaigns. This study aimed to determine current dehydration knowledge of caregivers of young children at risk for diarrheal illness. Three samples of caregivers of young children in a peri-urban community on the outskirts of Santo Domingo, Dominican Republic were recruited: a community sample (n=251), attendees of a nutrition rehabilitation program (n=223), and attendees of a general paediatric clinic (n=67). Caregivers participated in a structured interview. Responses were contrasted with commonly promoted prioritized dehydration signs. Thinness (42%), dry lips and/or mouth (33%) and sunken eyes (31%) were the most commonly reported signs across groups. The latter two correspond to prioritized dehydration signs. Various signs suggestive of under-nutrition were frequently mentioned as manifestations of dehydration (49%). Findings suggest some overlap in current caregiver knowledge and prioritized dehydration signs. Further research, including a qualitative approach, is needed to further explore caregivers' perceptions of dehydration in relation to under-nutrition.

115

EMCOUNTER: A NOVEL, DYNAMIC EPIDEMIOLOGIC SURVEILLANCE TOOL FOR THE DEVELOPING WORLD

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In recent years, the specialty of emergency medicine has gained tremendous interest in the developing world. Despite this, it remains a globally nascent specialty. Few countries have formally recognized the field, and even fewer have developed the training programs, infrastructure, legislation, and public education necessary to provide for a comprehensive approach to emergency care. Furthermore, there is little knowledge of what actually comprises medical emergencies in these settings. Current developmental and educational models use largely Western paradigms, which almost certainly differ from the unique needs and local variability of the rest of the globe. To address this knowledge gap, we developed Project EMcounter, a web-based tool capable of analyzing variations in the epidemiology and management patterns of medical emergencies in the developing world. The project makes novel use of an online data entry tool, functioning as a receiving database for the real-time collection of information from the paper charts of select emergency departments across the developing world. EMcounter was piloted at Sundaram Medical Foundation in Chennai, India from 2006 to 2008, during which data was collected on 13,214 separate patients, revealing important variations in disease patterns and practice constraints. However, far more than a static data collection tool, EMcounter has enormous potential as a dynamic real-time epidemic surveillance system. Because data is collected at hospital receiving rooms and emergency departments (often the first points of presentation for emerging epidemiologic trends), and given the real-time nature of the tool's data entry system, the tool is ideally suited to map epidemics and other current medical trends in select locations in the developing world. We are currently exploring this potential by developing an open-access web-based data visualization interface that allows users to modify data sets in order to graphically represent trends of interest. Next steps for the project include interfacing with electronic medical records to fully streamline the data entry process.

116

FETAL HEART RATE DURING ACUTE MALARIA

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To study the time course of the maternal and fetal heart rate (FHR) during recovery from acute malaria, we examined 40 pregnant women with acute malaria and 40 healthy pregnant women. Malaria patients were hospitalized until recovery with a minimum of 3 days. Healthy subjects were measured only once.

FHR during malaria was measured every 4hrs on the first day after initiating Arthemether lumefantrine (AL) treatment, and every 8 h for another two days. Maternal vitals were measured every 8 h for 3 days. The table shows the measurement on T0, of malaria patients and of healthy subjects (1 healthy Baseline measurements of malaria patients (n=40) compared to healthy women (n=39) were respectively: Gest. age (wks) 28.8 and 24.6 (p-value 0.006); upper FHR (bpm) 165 and 158 (p-value 0.054); lower FHR (bpm) 137.5 and 128.7 (p-value 0.016); mean blood pressure (mm Hg) 75 and 81 (p-value 0.001); pulse pressure (mm Hg) 40 and 42 (p-value 0.2); pulse rate (bpm) 109 and 81 (p-value <0.001); and Geometric mean. parasite/µl 13795. Complete time series were collected from 33 malaria patients. During recovery FHR normalized on average within 20 h. Maternal fever clearance was also 20 hrs but maternal heart rate normalized much later, after approx. 32 h. Maternal blood pressure was low and pulse rate was high in malaria patients whereas fetal heart rate(FHR) was elevated. In conclusion, the circulatory effects of acute malaria during pregnancy are compatible with decreased circulating maternal blood volume. In contrast, the FHR normalises at the same rate as the maternal body temperature.

117

IMPLICATING THE S1P PATHWAY IN CEREBRAL MALARIA PATHOLOGY

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Excess inflammatory responses as well as loss of vascular integrity have been implicated in cerebral malaria pathology. Sphingosine-1-phosphate is a tightly regulated signaling sphingolipid whose functions include

regulating endothelium homeostasis and inflammation. We hypothesized that S1P signaling is altered during infection and that this may contribute to endothelial activation and inflammation thought to play an important role in the pathogenesis of cerebral malaria. We found that extracellular plasma S1P levels were significantly decreased in Ugandan children with cerebral, but not uncomplicated, malaria. Using a murine model of experimental cerebral malaria, we also demonstrated that mice with reduced S1P lyase (an S1P degrading enzyme) activity, had higher survival rates compared to their wildtype littermates when infected with *Plasmodium berghei* ANKA (PbA). To further investigate the role played by S1P during infection, we treated mice infected with PbA with compounds that interfere with the S1P pathway and currently in human trials for other conditions (FTY720 or LX3305). Prophylactic treatment with either compound improved survival to PbA infection. However, with therapeutic administration, only FTY720 treatment proved beneficial. In animals having received prophylactic treatment with FTY720, we observed a decrease in IFNg and TNF levels both in plasma (protein) as well as the brain (mRNA). Vascular integrity was also improved in animals treated with FTY720 compared to untreated mice: (1) plasma protein levels of endothelial cell activation markers such as sICAM, and brain mRNA levels of ICAM were decreased, (2) Ang1 (a regulator of endothelial quiescence) plasma levels, as well as brain mRNA levels were increased, (3) Evans blue staining of the brain was also reduced. In summary, we present the first data implicating the S1P pathway in the pathogenesis of human and murine ECM and suggest that therapeutic manipulation of this pathway may represent a new adjunctive treatment strategy for severe or cerebral malaria.

118

ROLE OF CD47 AND SIRPA IN MALARIAL PATHOGENESIS

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CD47 engagement by macrophage SIRPα inhibits phagocytic activity and protects red blood cells (RBCs) from erythrophagocytosis. Conversely, decreased levels of CD47 expression are associated with an "eat me" signal and increased macrophage clearance of RBCs. Non-opsonized *Plasmodium falciparum*-parasitized RBCs are phagocytosed by macrophages but, CD47 expression on malaria infected RBCs has not been well studied. Based on the hypothesis that *P. falciparum* may modify RBC expression of phagocytic signals such as CD47, here we report, using enzyme immunoassay and flow cytometric assay, that CD47 expression is decreased on *Plasmodium*-parasitized RBCs at ring-stages ($P < 0.001$) and mature-stages ($P < 0.001$). We further show, that macrophages from SHP-1 knock-out mouse and macrophages treated with anti-SIRPα antibody (anti-CD172) enhance phagocytosis of ring-stage *falciparum*-parasitized RBCs. Finally, mice lacking CD47 and congenic controls were intrapenitonially inoculated with *P berghei* ANKA. At day 6 after inoculation the CD47-/- mice display significantly lower parasitemia ($P < 0.0001$) and survive longer ($P < 0.0001$) compared with their CD47-expressing littermates. These results support a potential role of CD47/SIRPα in protection against severe malaria.

119

MALARIA AND NON-TYPHOIDAL SALMONELLA: UNDERSTANDING THE UNDERLYING MECHANISMS OF HUMAN CO-INFECTION USING AN ANIMAL MODEL

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Co-infection of malaria and non-typhoidal Salmonella (NTS) is prevalent in endemic areas of sub-Saharan Africa. In children, co-infection results in severe disease. In particular, bacteremia resulting from NTS intestinal escape is much more frequent in malaria-infected children. We have recapitulated this phenomenon in a murine model to investigate the nature and location of the barrier defect in containment of NTS. In human malaria infection, intestinal permeability is increased, suggesting that bacteremia results from intestinal escape of NTS. Two factors may contribute to malaria-associated intestinal permeability: parasite sequestration in the intestinal vasculature and parasite-induced L-arginine deficiency. Hypoargininenemia is a hallmark of malaria infection, reducing the synthesis of anti-parasite nitric oxide (NO) and exacerbating restricted blood flow by parasite sequestration. Further, L-arginine-related genes are induced in the gut during the early stages of NTS infection, and we can infer that L-Arginine depletion may be occurring in the intestinal response to NTS infection in a non-human primate model. Based on these observations and the knowledge that oral L-arginine supplementation can restore intestinal barrier function, we hypothesized that L-arginine deficiency in co-infected mice contributes to NTS escape from the intestine. To test this hypothesis, we supplemented *Plasmodium* yoelii nigeriensis-infected mice with oral L-arginine and monitored bacterial translocation from the intestine and peripheral parasitemia. As shown previously, parasite infection resulted in increased bacterial translocation in the liver, spleen and Peyer's patches over uninfected controls. However, parasite-infected mice supplemented with oral L-arginine showed significantly decreased bacterial translocation in the mesenteric lymph node when compared to non-supplemented infected mice. We found higher levels of L-citrulline in the serum of supplemented mice, suggesting that oral L-arginine is metabolized by NO synthase as opposed to arginase. These data suggest that oral L-arginine may have restorative or reparative effects on malaria-induced pathology. Our murine studies should help to characterize the mechanisms of co-infection pathology, to aid in the identification of new drug targets, and to enhance currently available therapeutics.

120

EFFECT OF CHRONIC SCHISTOSOMIASIS ON SEVERE MALARIA IN A PRIMATE MODEL

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Malaria and helminth infections are the two most prevalent parasitic diseases globally with annual mortality of about 500 million in the former and approximately 2 billion people infected with helminths. Since the epidemiological distribution of both diseases overlap, concomitant infections are a common occurrence. Studies on the effect of one disease on the other in mouse models and humans, show conflicting results due to differences in mouse strain, intensity of helminth infection, age of population and differing study designs. In order to provide proof of principle, this study hypothesized that chronic schistosomiasis results in delay in the onset and severity of malaria.We used the Olive baboon (Papio anubis) as a study model since they are natural hosts of *S. mansoni* with the capacity of harboring a substantial schistosome infection that is long-term, unlike mice. The baboon also presents a model of *Plasmodium knowlesi* infection that is useful in studying uncomplicated and severe malaria with cerebral involvement, and which is now recognized as the fifth malaria parasite of humans. Four groups of baboons were used. Groups A, B (n=8) and D (n=3) were infected with 500 *S. mansoni* cercarie, and the disease was left to progress to the chronic phase. To determine the effect of treatment on co-infection, group A was treated with praziquantel at week 14 and 15 post infection. Four weeks later, groups A, B and C (n=8) were inoculated with 1x105 *P. knowlesi* parasites. Baboons were monitored daily and clinical parameters recorded. Sera and PBMCs were collected at baseline, before and after treatment and at end-point to determine humoral and cellular immunological responses. Results showed that animals infected with *P. knowlesi* had an early onset of parasitemia and succumbed to severe malaria unlike majority of baboons with co-infection. Comparative assessment of immunological and clinical

parameters will be presented in detail. This study shows that presence of schistosomiasis in malaria infected animals' results in delay in the onset and severity of acute malaria

121

THE IMPORTANCE OF HEMOGLOBIN LEVEL AT ENROLLMENT ON SUBSEQUENT MALARIA RISK: RESULTS FROM A PEDIATRIC COHORT IN MALI, WEST AFRICA

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WHO estimates around 60% of people (especially children <5 years old) living in malaria-endemic areas of Africa have iron-deficiency anemia. Studies to evaluate the effect of iron supplementation on malaria risk have produced controversial results. Further, the impact of baseline hemoglobin (Hb) level on subsequent risk of malaria has not been well documented or routinely assessed. We initiated a 5-year longitudinal cohort study in three villages in rural Mali. From June 2008 to December 2009, we enrolled 1419 children aged 6 months to 17 years. We enrolled children just prior to the 2008 malaria transmission season (N=1258) or at 6 months of age (N=161). Each child's age, ethnicity, village, Hb level, and ABO blood type were recorded and the presence of sickle HbS, HbC, alpha-thalassemia, and G6PD deficiency determined. In 1356 children with complete data, we diagnosed 1933 episodes of malaria (90% uncomplicated) during 2 consecutive annual transmission seasons. The relative risk (RR) for each factor was calculated by a Poisson regression model. In the model, we divided children into three groups based on Hb level (Hb<8.5 g/dL, N=82; 8.5-12 g/dL, N=877; >12 g/dL, N=397). As expected, older children had lower risk of malaria than younger children. Taking into account all covariates, we found that children with Hb >12 g/dL had significantly lower risk (RR 0.81, 95%CI 0.69-0.95, p =0.008) of malaria than those with Hb 8.5-12 g/dL. Interestingly, children with Hb <8.5 g/dL and Hb 8.5-12 g/dL did not differ in malaria risk. HbS was the only other factor associated with significant change in RR. The reduced malaria risk in children with Hb >12 g/dL was not associated with reduced parasite densities (adjusted geometric mean of the Hb >12 g/dL group was 0.92 times that of the Hb 8.5-12 g/dL group, 95%CI 0.63-1.34, p=0.660). Our data indicate that baseline Hb level may be an important host factor (and thus a major confounder) in natural history and interventional studies which measure malaria incidence as a primary outcome. Further work is needed to determine if relatively high Hb levels are directly involved in the mechanism of protection or whether an unmeasured factor that correlates with high Hb levels confers protection from malaria.

122

PLASMODIUM CHABAUDI INFECTION FOLLOWED BY *P. BERGHEI* INFECTION IN C57BL/6 MICE PROVIDES NOVEL MURINE MODEL FOR STUDYING SEVERE MALARIAL ANEMIA

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Severe malarial anemia from *Plasmodium falciparum* claims the lives of thousands of children in sub-Saharan Africa every day. The pathogenesis of this anemia is not well understood and progress has been hampered by the lack of an inexpensive and reproducible animal model. We aimed to develop a relevant model of severe malarial anemia using C57BL/6 mice. Infection of these mice with *Plasmodium berghei* ANKA is uniformly fatal whereas infection with *P. chabaudi* AS leads to severe anemia with high parasitemia followed by full recovery. We determined that infection with *Plasmodium berghei* ANKA following recovery from a *Plasmodium chabaudi* AS infection resulted in anemia with a low level parasitemia (<10%). Mice developed splenomegaly and hepatomegaly with histological evidence of erythrophagocytosis in the liver. Inflammatory cytokines IL-12 and TNF-α were significantly elevated over those in naïve mice infected with *P. berghei*. This new mouse model provides a highly reproducible and relevant platform for studying host and parasite factors that contribute to the pathogenesis of severe malarial anemia.

123

MODULATION OF MEMBRANE AND SOLUBLE TREM-1 IN MALARIA INFECTION

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Excessive or dysregulated host pro-inflammatory responses to malaria infection have been implicated in the pathogenesis of severe disease. A number of innate immune components have been shown to contribute to these responses, including Toll-like receptors (TLR), although the full complement of host inflammatory pathways remains to be characterized. Triggering receptor expressed on myeloid cells-1 (TREM-1) is a germline receptor on monocytes and neutrophils that is upregulated upon TLR stimulation. TREM-1 synergizes with TLRs to induce inflammation, and has been found to play a role in sepsis pathophysiology. We hypothesized that TREM-1 expression is modulated during malaria infection and that TREM-1 may contribute to disease severity. We exposed human peripheral blood mononuclear cells (PBMCs) to *Plasmodium falciparum*-infected red blood cells (RBCs) or uninfected RBCs *in vitro*. Incubation of PBMCs with malaria-infected RBCs for 24 hours resulted in a significant decrease in TREM-1 surface levels on monocytes (p=0.018), and induced release of soluble TREM-1 (sTREM-1), which is thought to be generated by cleavage of membrane TREM-1. We next examined TREM-1 expression in the *P. berghei* ANKA model of experimental cerebral malaria. TREM-1 mRNA expression in the brain was elevated in mice on Day 6 of infection compared to uninfected mice (p<0.05). Finally, we measured sTREM-1 in the plasma of pediatric malaria patients in a case-control study in Uganda. Plasma sTREM-1 levels at admission were significantly elevated in severe malaria patients compared to uncomplicated cases (median (range) in pg/mL: uncomplicated 154.9 (44,1519) vs severe 371.5 (72.7,2428); p<0.0001), and were higher in fatal cases of severe malaria compared to survivors (survivors 324.6 (72.7,1321) vs fatal 528.7 (244.3,2428); p=0.0021). In summary, we show that TREM-1 is modulated during malaria infection. We are currently investigating whether TREM-1 signaling contributes to the pathogenesis of severe malaria.

124

THROMBOCYTOPENIA IN PATIENTS WITH *PLASMODIUM VIVAX* IN A COLOMBIAN ENDEMIC AREA

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Malaria remains an important health problem in tropical countries like Colombia. The malaria has clinical manifestations that can change from short duration fever episodes, if the diagnosis is opportune and the treatment is effective, up to systemic severe complications and death. The hematic changes associated with malaria are well recognized, but the specific changes can to vary according to the endemicity levels of the malaria, history of hemoglobin disease, nutritional condition, demographic factors and malaria immunity. The proposal of this study was to determine the behavior of the platelets in patients with *Plasmodium vivax*. Venous

blood was collected in K2EDTA Vacutainer® tubes for automatized platelet count; additionally carry out thin and thick smear from peripheral blood from 200 individuals (100 with *Plasmodium vivax* and 100 control individuals of the same area). Thrombocytopenia was defined as platelet count under 150,000/µL. Results show that 67% of patients with *P. vivax* had thrombocytopenia. The platelets average in healthy population was 278.000/uL and 125.000/uL for the patients. Platelets average was 84.500/uL in patients with thrombocytopenia, in these patients the age average was 33 years old and they had a normal Body Mass Index. Male (64%) shows more frequency of thrombocytopenia that female (36%). Mean of parasitemia in patients with *P. vivax* and thrombocytopenia was 4.040 parasite/uL. Did not observe relationship between parasitemia and thrombocytopenia. Neither is clear the relationship between previous episodes of malaria and thrombocytopenia. This study suggests that in this population the thrombocytopenia is a frequently finding in patients with *Plasmodium vivax*. The clinician should consider malaria in patients with fever syndrome and thrombocytopenia when thinking in dengue. Is important to direct future studies to the virulence of the parasite and the immune response in this individuals, for understand the mechanism involved in the decrease of platelets overall in patients with low parasitemia.

125

WHAT IS A HYPNOZOITE?

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In 1980, Krotoski and his colleagues reported their important discovery of a uninucleate, apparently dormant stage in the life cycle of *Plasmodium*. Two years earlier, the suggested use of the term "hypnozoite" had been set out by me in a key paper (reprints of which are still available) that has remained obscure because of the journal in which it appeared. In relation to the prevailing hypnozoite concept, the information to be presented at this meeting is, therefore, dramatically new. Following non-malarial work carried out in the 1970s while I was a PhD student at Imperial College London, the implications of the results of my research if extrapolated to the incompletely elucidated plasmodial life cycle, were pointed out; and I named the dormant, sporozoite-like apicomplexan (sporozoan) form. At that time, the concept of the occurrence of dormant malarial parasites in the liver and/or elsewhere was still a hypothetical idea. Very few people are aware that the "hypnozoite" has been "defined". This was done at some length in the abovementioned publication. It was proposed that the word "hypnozoite" be used for dormant stages that might in the future be found in the life cycle of *Plasmodium* (which has since happened). Moreover, it was explained (*inter alia*) that "hypnozoite" would also describe post-divisional, dormant, sporozoite-like apicomplexan forms that are not biologically or ultrastructurally typical merozoites (leaving aside a possible good example); as well as dormant sporozoites in the life cycle of *Isospora* (*Cystoisospora*), for instance. Although the paper concerned was published more than three decades ago, the analysis is still valid today. In summary, just as "merozoite" and "sporozoite" are not exclusive to *Plasmodium*, the descriptive name "hypnozoite" is not only applicable to dormant liver stages of *Plasmodium*, but (contrary to current general understanding of the use of the term) to some other dormant apicomplexan forms as well.

126

MINIMALLY INVASIVE POST MORTEM TISSUE SAMPLING: A PROTOCOL TO ELUCIDATE HOST-PARASITE INTERACTIONS IN TROPICAL AREAS

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Parasitic diseases are a major cause of death in tropical areas; however, pre-mortem clinical diagnoses are challenging and the in vivo mechanisms are incompletely understood. For example, respiratory distress due to malaria is difficult to distinguish from viral or bacterial causes and the role of parasite sequestration is of uncertain significance. We are developing a minimally invasive, culturally acceptable, easily implemented, safe and rapid autopsy procedure that will allow extensive tissue sampling after death due to infection in resource poor communities such as rural Africa. Using an autopsy cohort at the University of Washington, we utilize a combination of in house manufactured and commercially available laparoscopic surgical tools. We have optimized sampling of lung, liver, spleen, brain and bone marrow. In this cohort, respiratory diseases have been well-represented, including cytomegalovirus pneumonitis, H1N1 influenza, and mycobacterium infection. Messenger RNA levels of vascular endothelial growth factor (VEGF) and other potential biomarkers tied to clinical syndromes are being assessed by quantitative PCR. In future field studies of malaria, we anticipate that the post mortem recovery of parasite and host material will assist in the identification of novel diagnostic markers and further elucidate host-parasite interactions related to severe disease.

127

ELEVATED SFLT-1 AND DECREASED VEGF LEVELS ARE ASSOCIATED WITH MALARIA-RELATED RESPIRATORY DISTRESS IN TANZANIAN CHILDREN

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Soluble fms-like tyrosine kinase 1 (sFlt-1), an inhibitor of vascular endothelial growth factor (VEGF), is elevated during placental malaria and has been implicated in the pathogenesis of acute respiratory distress syndrome. Prior studies have found that during malaria in non-immune adults, levels of VEGF are elevated and sFlt-1 is unchanged, whereas during pediatric malaria in endemic areas, levels of VEGF are decreased and correlate with severity of disease. We hypothesize that sFlt-1 may contribute to angiogenic imbalance during malaria-associated respiratory distress in endemic areas. Here we report that for children living in Muheza, Tanzania, a high transmission area, uncomplicated malaria was associated with decreased plasma VEGF levels by ELISA, and that malaria-associated respiratory distress was associated with further decreased VEGF level. Plasma sFlt-1 was inversely correlated with VEGF, and sFlt-1 levels were significantly increased during malaria-associated respiratory distress. Paradoxically VEGF expression *in vitro* was stimulated ~20-fold by co-culture of *P. falciparum*-infected erythrocytes with peripheral blood mononuclear cells. This suggests that systemic VEGF levels in the host may be derived from non-hematopoietic tissue and that malaria may have opposing local versus systemic effects on VEGF expression, which may explain some of the differences seen between adults and children with malaria. We previously characterized a microsatellite polymorphism in the 3′ untranslated region of FLT1 that was associated with outcome during placental malaria. We are currently assessing this genotype in relation to outcome during pediatric malaria. These data suggest that sFlt-1 is

associated with decreased circulating free VEGF levels and contributes to the pathogenesis of malaria-associated respiratory distress in endemic areas.

128

MALARIA PARASITE *PLASMODIUM FALCIPARUM* DD2 SPONTANEOUSLY SWITCHES FROM SIALIC ACID-DEPENDENT TO SIALIC-ACID INDEPENDENT ERYTHROCYTE INVASION IN SUSPENSION CULTURE

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Plasmodium falciparum parasites can broadly be classified as sialic acid (SA)-dependent or SA-independent based on their ability to invade neuraminidase-treated erythrocytes. *P. falciparum* Dd2 is classified as SA-dependent since it usually invades neuraminidase-treated erythrocytes at <10% of the rate of invasion of untreated erythrocytes. We compared the rates of SA-independent invasion in two parallel Dd2 cultures, one incubated with gentle shaking (*Suspended*) and another without shaking (*Static*). While the ability of Dd2 *Static* to invade neuraminidase-treated cells remained below 10% relative to invasion of untreated erythrocytes, SA-independent invasion increased steadily in Dd2 *Suspended* over time, reaching about 50% after 12 weeks in culture. Interestingly, this switch in invasion phenotype was reversible, such that when Dd2 suspended was returned to static conditions, there was a gradual loss in its ability to invade neuraminidase-treated erythrocytes. These observations appear to be unique to Dd2 since two other *P. falciparum* strains, 7G8 and FVO, did not significantly alter their invasion patterns when cultivated in suspension for a similar length of time. Targeted gene expression analyses revealed that some known *P. falciparum* proteins were upregulated several hundred-fold in Dd2 *Suspended* compared to Dd2 *Static*, including PfRh4, which has been shown to be involved in SA-independent invasion. Additional genome-wide microarray experiments are currently being performed to further investigate the molecular changes that are responsible for the changes in Dd2 invasion patterns in suspension cultures. Our observations in suspension cultures are similar to those obtained when Dd2 switches to Dd2NM after continuous culture in neuraminidase-treated erythrocytes. Thus, our investigations offer new opportunities for examining the mechanisms of PfRh4 regulation, and for identifying the elusive parasite ligands that mediate SA-independent invasion which remain critical for the design of any successful invasion-blocking vaccine strategies.

129

PCR-BASED POOLING OF DRIED BLOOD SPOTS FOR DETECTION OF MALARIA PARASITES: OPTIMIZATION AND APPLICATION TO A COHORT OF UGANDAN CHILDREN

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Sensitive, high-throughput methods to detect malaria parasites in low transmission settings are needed. PCR-based pooling strategies may offer a solution. We first used laboratory prepared samples to compare 2 DNA extraction and 4 PCR detection methods across a range of pool sizes and parasite densities. Pooled Chelex extraction of DNA followed by nested PCR of cytochrome b was the optimal strategy, allowing reliable detection of a single low parasitemic sample (100 parasites/μL) in pool sizes up to 50. This PCR-based pooling strategy was then compared with microscopy using 891 dried blood spots from a cohort of 77 Ugandan children followed for 2 years in a low endemic urban setting. Among 419 febrile episodes, 35 cases of malaria were detected using the PCR-based pooling strategy and 40 cases using microscopy. All five cases of malaria not detected by PCR were from samples stored >2 years with parasitemia < 6000/μL, highlighting the issue of possible DNA degradation with long-term storage of samples. Among 472 samples collected in asymptomatic children as part of routine surveillance, 15 (3.2%) were positive by PCR-based pooling compared to 4 (0.8%) by microscopy (p=0.01). Thus, this PCR-based pooling strategy for detection of malaria parasites using dried blood spots offers a sensitive and efficient approach for malaria surveillance in low transmission settings, enabling improved detection of asymptomatic submicroscopic infections and dramatic savings in labor and costs.

130

THE PREDICTIVE VALUE OF RAPID DIAGNOSTIC TESTS FOR GAMETOCYTEMIA IDENTIFIED BY RT-PCR

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Accurate diagnostic tools for malaria are essential to ensure that infections are not missed. In Zambia, rapid diagnostic tests (RDTs) are the main method used to diagnose malaria. The use of RDTs is important for clinical purposes, but the presence of gametocytes are the critical parasite stage for sustaining malaria transmission. Identification of gametocyte carriers will be important for malaria elimination. Our objective was to assess the association between the presence of gametocytes detected by RT-PCR and RDT positivity to provide insight on the proportion of gametocyte carriers identified by RDTs. A cross-sectional survey of individuals residing in randomly selected households was conducted in Mapanza, Choma District, in southern Zambia throughout 2008. In total, 309 blood samples were collected and tested using both RDT and RT-PCR, the latter on samples stored as dried blood spots. The RDT was an ICT Malaria P. f cassette, based on an antibody to the histidine-rich protein 2 antigen of *Plasmodium falciparum* (pfHRP-2) expressed by asexual stages. The RT-PCR detected the pfs25 mRNA expressed in *P. falciparum* gametocytes. Of the 309 individuals, 31 (10%) were RDT positive, 14 (4.5%) were RT-PCR positive for gametocytes and 9 (2.9%) were positive by both methods. Almost half (45%) of the RDT positive individuals also tested positive for gametocytes. Of the gametocyte positive individuals, 64% were RDT positive. As the first line treatment for malaria in Zambia is artemether-lumefantrine (Coartem®), which has activity against gametocytes, treatment of RDT positive, asymptomatic persons may impact malaria transmission by reducing gametocytemia. However, one third of gametocyte carriers were not detected using RDTs, highlighting the need to identify these carriers through alternative methods to achieve malaria elimination.

131

EVALUATION OF A RAPID DIAGNOSTIC TEST OF MALARIA (HISTIDIN RICH PROTEIN 2) IN A PAEDIATRIC HOSPITAL IN DAKAR, SENEGAL

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HRP2- based Rapid diagnostic test is one of the malaria RDTs which can ensure a rational use of ACTs. The HRP2 antigen is a hydro-soluble

protein which can go through the spinal fluid. This study aimed to test the sensitivity and the specificity of these tests in paediatric hospital areas in peripheral blood and to search the HRP2 in the spinal fluid. An exploratory study was conducted into Albert Royer paediatric hospital in Dakar from November 2006 to May 2007. All patients less than fifteen years presenting clinical symptoms of malaria were included. A thick drop and commercial RDT Paracheck*PF was performed and for patients with neurological symptoms, RDT was performed on spinal fluid. Of the 1223 screened patients, 137 were found positive by the RDTs and 136 by the thick drop. The sensitivity and specificity are respectively 98,5% and 99,7%. The positive and negative predictive values were respectively 97,8% and 99,8%. Antigen HRP2 was never detected in the spinal fluid on the 107 patients with clinical severe cases. In conclusion, the paracheck applied to blood in the children is thus very sensitive and specific to *Plasmodium falciparum*. The HRP2 RDT is more sensitive among patients presenting severe malaria than those presenting uncomplicated malaria. Antigen HRP2 not found in the spinal fluid was probably either due to its molecular weight, or because the RDTs unable to detect it. The paracheck*pf is a useful tool for malaria diagnosis but microscopic examination remains the standard method.

132

QUANTITATIVE MAGNETIC FRACTIONATION FOR *PLASMODIUM FALCIPARUM* GAMETOCYTE DETECTION

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A method for quantitative analysis of the gametocyte density in human blood samples is presented. This method is based on magnetic fractionation using commercially available magnetic fractionation columns and exploiting the magnetic susceptibility of mature *Plasmodium falciparum* gametocytes. The quantitative approach utilizes magnetic microspheres as a calibration standard, which are added to each blood sample at a known concentration. Gametocytes and magnetic microspheres are captured simultaneously inside the magnetic fractionation columns. The magnetically captured material can be eluted from the columns, placed on a microscope slide and stained according to standard protocol. By counting gametocytes and the magnetic microspheres after magnetic fractionation, the original gametocyte density in the blood samples can be determined from their ratio. The limits of quantification for the presented method were determined from serial dilutions with known gametocyte density. The upper limit of quantification of this method is above 103 gametocytes per mL, where quantitative analysis of the slides became impossible due to an overabundance of observed gametocytes. The lower limit of quantification was determined to be less than 1 gametocyte per mL of blood and was characterized by a departure of the standard curve from linearity. The lower limit of detection for *P. falciparum* gametocytes using this method lay in the range of 0.01-0.1 per mL.

133

VARIATION IN THE EXPRESSION OF DIAGNOSTIC BIOMARKER HISTIDINE RICH PROTEIN II (HRP2) IN COLOMBIAN *PLASMODIUM FALCIPARUM* ISOLATES

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More than half of the Colombian population is at risk of contracting malaria. Most of the fatal cases occur because of complications due to delays in diagnosis and treatment. At present, the gold standard diagnosis method for malaria is based on microscopy; but its use in remote endemic areas is restricted by lack of qualified personal and basic infrastructure. Such drawbacks have led to the development of simpler diagnostic strategies, including Rapid Diagnostic Tests RDTs. Most RDTs available use HRP2 as a target; nevertheless, it has been reported that sequence variations of HRP2 affect its sensitivity. At present there is not enough evidence about HRP2 variability in Latin American isolates and its relationship with RDT performance. The aim of this study was to evaluate the amount of HRP2 present in *Plasmodium falciparum* isolates from two endemic cities in the Colombian Pacific coast, in order to determine possible differences between locations and their effects on RDT performance. Twenty-three blood samples from patients with malaria-*falciparum* from Buenaventura and Tumaco, cities located in the Colombian Pacific coast, were assessed by measurement of HRP2 concentration using ELISA-HRP2, RDTs and thick smear. Statistical analysis revealed association between RDT performance and HRP2 concentrations. A slight variation, although without statistical significance, was found in HRP2 antigen levels between study sites, as well as a large variation in antigen concentrations of samples with the same parasitaemia. In contrast to previous reports, there was no correlation between initial parasitaemia and HRP2 concentration, suggesting difference in HRP2 production between parasites. Our results indicate that not only the pfHRP2 antigen sequences, but also the antigen expression levels should be studied more carefully in various endemic areas of the country, as variations in both could have significant consequences on the performance of malaria RDTs.

134

DETECTION OF *FALCIPARUM* GAMETOCYTES USING A REVERSE TRANSCRIPTION-LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP)

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Plasmodium falciparum gametocytes are usually present in peripheral blood at a very low level. Therefore, a sensitive assay is needed for gametocyte detection. In this study, loop-mediated isothermal amplification (LAMP) was developed for clinical detection of *P. falciparum* gametocytes. The transcripts of Pfs16 for sexually committed rings and Pfs25 for mature gametocytes were detected by reverse transcription (RT)-LAMP using 82 clinical blood samples. To evaluate an RT-LAMP assay, nested reverse transcription (RT)-PCR was used as a gold standard. RT-LAMP demonstrated a detection limit of 1 parasitized red blood cell (RBC)/500 µl of blood for both Pfs16 and Pfs25. RT-LAMP detected Pfs16 in 30 of 30 samples positive by nested RT-PCR (100% sensitivity) and 1 in 52 samples negative by nested RT-PCR (98.1% specificity). For Pfs25, RT-LAMP detected 15 of 15 samples positive by nested RT-PCR (100% sensitivity) and none of 67 samples negative by nested RT-PCR (100% specificity). The negative predictive value (NPV) and positive predictive value (PPV) of RT-LAMP for the detection of Pfs16 were 100% and 96.8%, respectively. The NPV and PPV for Pfs25 were 100%. Collectively, compared to nested RT-PCR, RT-LAMP had a higher sensitivity and a similar specificity with a shorter assay time. Since RT-LAMP requires solely basic instruments and the result inspection can be done by visualization, RT-LAMP developed here enable a simple and reliable test for analysis in molecular epidemiological study in malaria transmission and gametocyte-targeted control.

135

EVALUATION OF REVERSE-TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP) FOR *PLASMODIUM FALCIPARUM* GAMETOCYTE DETECTION IN ENDEMIC AREA OF THAILAND

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Gametocytes are usually present in peripheral blood at a low level. Therefore, a sensitive assay is needed for gametocyte detection. In this study,reverse-transcription loop-mediated isothermal amplification (RT-LAMP) was developed for clinical detection of *Plasmodium falciparum* gametocytes. The transcripts of *P. falciparum* surface antigen 16 (*Pfs*16)

for sexually committed rings and *Pfs*25 for mature gametocytes were detected by RT-LAMP using 30 microscopically *falciparum*-positive blood samples collected from Mae Sot and Mae Kasa, Tak, the North western of Thailand. To evaluate RT-LAMP assay, these samples were tested by reverse transcription-polymerase chain reaction (RT-PCR). The sensitivities of RT-LAMP for *Pfs*16 and *Pfs*25 detection were 105 and 10 times higher than those of RT-PCR and nested RT-PCR, respectively. Among 30 samples, 3.3% was RT-PCR-positive for *Pfs*16 and *Pfs*25. Contrastingly, 56.7% and 40% were RT-LAMP-positive for *Pfs*16 and *Pfs*25, respectively. RT-LAMP provided similar specificity but higher sensitivity as compared to those of RT-PCR and nested RT-PCR with shorter assay time and does not required DNA purification. Collectively, this study indicates that RT-LAMP is a highly sensitive, reliable, and user-friendly method in gametocyte detection applications. RT-LAMP developed here can be useful for the epidemiological study in malaria transmission and gametocyte-targeted control.

136

IDENTIFICATION OF SENSITIVE MALARIA RDTS SUITABLE FOR THE DETECTION OF PFHRP2 NEGATIVE *PLASMODIUM FALCIPARUM* INFECTIONS IN THE PERUVIAN AMAZON

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Malaria RDTs are playing an increasing role in malaria control, especially in remote settings where good microscopy is difficult to maintain or unavailable. Recent findings showed a high proportion of *Plasmodium falciparum* parasites lacking the HRP2 gene in Peru, pointing to the need of sensitive RDT that can detect them. We compared the performance of 2 RDTs detecting pfHRP2/panLDH (First Response Malaria Antigen Combo pLDH/HRP2, Premier Medical Corporation) and pfLDH/panLDH (Advantage Mal Card, J Mitra & Co), for *P. falciparum* diagnosis in the Peruvian Amazon. From November 2009 to March 2010, symptomatic patients with microscopically confirmed *P. falciparum* infections were enrolled in two study arms: i) Active Case Detection (ACD) in 5 remote sites of the Loreto Region, and ii) Passive Case Detection (PCD) in 2 Health Centers nearby Iquitos and Yurimaguas. Each patient was tested with the two above mentioned RDTs. 31 and 44 patients were enrolled by PCD and ACD, respectively, with a significant proportion of HRP2 negative *P. falciparum* infections (PCD: 29.0%, ACD: 33.0%) being detected by microscopy and by the pfLDH line of Advantage Mal Card but not by the pfHRP2 line of First Response RDT. The sensitivities of First Response and Advantage Mal Card RDTs were 71.0% vs 97.1% for samples collected by PCD, and 65.0% vs 100% for samples collected by ACD, but raised up to 100% vs 97.05% (PCD), and 100% vs 100% (ACD), respectively, when excluding HRP2 negative samples. In conclusion, the main cause of the observed differences in sensitivity was the high prevalence of *P. falciparum* HRP2 negative parasites, being detected by First Response RDT as non-*P. falciparum* infections. In the Peruvian context, this leads to a misdiagnosis as *P. vivax* and to dispensing of drugs with poor efficacy against *P. falciparum*. These results corroborate previous reports indicating that HRP2-based RDTs may not be appropriate for *P. falciparum* diagnosis in the Peruvian Amazon, and should therefore be considered if RDTs are to be used in the country.

137

DETECTION OF SINGLE RING STAGE *PLASMODIUM FALCIPARUM* IN HUMAN THIN FILM BLOOD SMEARS USING FTIR MICROSPECTROSCOPY AND DIFFERENTIATION OF PLASMODIUM POSITIVE FROM PLASMODIUM NEGATIVE RED BLOOD CELLS

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Currently, rapid diagnostic tests for malaria infection perform poorly at low parasite loads, are degraded by severe temperatures, and contain reagents, which contribute to their costs. The overall objective of this study was to perform a preliminary evaluation of the utility of FTIR microspectroscopy for *in vitro* diagnosis of thin film blood smears for malaria infection. FTIR microspectroscopy has potential advantages in detecting low parasite loads, is not affected by temperature, and does not require any reagents. Geimsa-stained thin film blood smear slides were analyzed in this study. 240 slides with ring stage *Plasmodium falciparum* infected human blood were prepared from culture. *P. falciparum* negative controls included 80 clinical *P. vivax* slides (collected and verified by expert microscopy (EM) and Polymerase Chain Reaction (PCR)), 40 slides with *Salmonella*- infected human blood (prepared from culture), and 40 uninfected human blood slides. Infrared spectra were measured from a small area of each slide (~12 microns x 12microns) usually containing only one red blood cell. Algorithms were written to differentiate *Plasmodium* positive spectra from *Plasmodia* negative spectra and tested by cross-validation. The sensitivity was 98.8% to 100% and the specificity was 95.4% to 100% for *Plasmodia* positive samples with a 95% confidence interval. These results suggest that further study of FTIR spectroscopy as an automated reagent-less diagnostic method with potential for detection of single parasites is warranted. Infrared spectroscopy could radically lower marginal test costs by eliminating the need for expensive consumables.

138

RAPID PARASITEMIA DETERMINATION BY FLOW CYTOMETRY USING A DNA-BINDING FLUORESCENT DYE

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Although the determination of parasitemia by light microscopy with Giemsa staining still remains as the golden standard in malaria diagnosis, this method becomes very laborious when the number of samples increases. To set up a high-throughput assay system of *Plasmodium falciparum* malaria, we tried to use flow cytometry (FACS) using PicoGreen that binds to double-strand DNA, and compared the results obtained by light microscopy and FACS. The two methods yielded fairly concordant parasitemias, the latter being more reproducible and faster than the former. For two samples or less, the microscopic method was faster, but FACS was faster for more than three samples. With the latter method, parasitemias of 200 samples could be determined by one person in about 6 hours including sample preparation steps when 50,000 cells per sample were read. Because the parasitemia values by the FACS method included some background noises of 0.2-0.5%, however, samples had to be treated with RNase prior to PicoGreen staining. Although this step was helpful in reducing the background to some extent, approximately one third of it persisted presumably due to mitochondrial DNA of reticulocytes. Under our assay condition, the dilution factor of PicoGreen over the range of 1/2,000 and 1/200,000 resulted in almost similar parasitemias.

139

PREVALENCE AND TRANSMISSION PATTERN OF *PLASMODIUM FALCIPARUM* INFECTION IN OSOGBO METROPOLIS, SOUTHWEST, NIGERIA

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Plasmodium falciparum malaria is an endemic disease especially in tropical areas with heavy rainfall that spread round the year. We therefore sought to investigate the prevalence pattern and clinical presentation of *falciparum* malaria in Osogbo and level of degree of prevalence were assessed and screened for *Plasmodium falciparum* infection by clinical assessment and microscopy using both thick and thin blood smears over a period of 12 months- August 2004 and July 2005. The prevalence of *Plasmodium falciparum* infection was found to be 52.8% with 341/646 of the patients been positive for *P. falciparum* parasite based on microscopy. Three hundred and five (47.2%) were aparasitaemic of which 162 (25.1%) had bronchopneumonia, 99 (15.3%) had upper respiratory tract infection, 32 (5.0%) had gastroenteritis and 12 (1.9%) had Otitis media. Between August and November 2004, 250 patients were screened and 160 (57.6%) of these patients were positive, while 180 patients were screened between December 2004 and March 2005 and 51 (28.3%) were positive. Between April 2005 and July 2005, 216 patients were screened and 130 (60.2%) of the patients were positive. When compared, the differences in the percentage of patients with positive microscopy in December to March with April to July and August to November were found to be significant ($P < 0.0001$), whereas the percentage difference in patients with positive microscopy in August to November and April to July was not significant ($P = 0.442$). The result of this study clearly shows that there are two distinct peaks of malaria transmission pattern in consonance with the rainfall pattern in the area.

140

QUANTIFYING AND MODELLING CROSS-BORDER HUMAN POPULATION MOVEMENTS INTO KENYA IN RELATION TO MALARIA INFECTION MOVEMENTS

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High levels of *Plasmodium falciparum* malaria transmission are found in certain areas of Kenya, principally areas bordering Uganda around Lake Victoria and bordering Tanzania at the coast. Cross-border movement is hypothesized to play a part in maintaining pockets of high transmission and human movement from such areas to regions of lower or zero transmission are likely to make malaria control and elimination challenging. These difficulties justify quantitative investigation of patterns and rates of population movement and infections they carry in and out of high transmission areas. This research draws on a wide range of data sources to investigate the role of human movement in driving transmission in these areas. Micro-census data, travel history surveys, air passenger flows, settlement location and sizes, cross-border crossings and road traffic datasets have been assembled to explore the range of population movements seen across Kenya and how they may vary by malaria season, region and demographic characteristics of the population. These will then be integrated with existing spatial datasets for Kenya, including demographic data, malaria endemicity maps and detailed transport networks to build network-based meta-population models of human and parasite movements, and to explore the likely effects of these movements on differing control policy scenarios. To understand population movement patterns, meta-population gravity models over a range of spatiotemporal scales have been developed, which will be fitted to the movement data and be used to predict movement patterns in parts of the country where data is less readily available. Linking these models with recently developed malaria transmission maps and simulation models allows assessment of malaria dispersal across Kenya and surrounding regions. The malaria transmission model used is an individual-based simulator, developed by the Malaria Atlas Project. The framework developed permits human movement to be incorporated and its implications of these to be assessed. The models will form evidence-based tools for malaria control planning in Kenya and whilst focus will remain on Kenya, methodologies developed will ultimately have strong relevance and application to other malaria endemic areas across the globe and for the study of other infectious diseases.

141

COMPLICATED *VIVAX* MALARIA AT A REFERENCE CENTRE IN THE BRAZILIAN AMAZON

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In South America, around 80% of Malaria episodes are caused by *Plasmodium vivax*. Although formerly seen as a benign condition, an increasing number of reports have changed this perception, pointing to a wide-array of complications associated with infection by this parasite. The objective of this study was to to describe the clinical manifestation of complicated *P. vivax* infection at a tertiary-reference centre in the Brazilian Amazon. All the inpatients with *P. vivax* infection evidence admitted at the Tropical Medicine Foundation of Amazonas at Manaus underwent a thorough clinical evaluation and additional complementary exams (several blood analysis,urine, feces and radiological tests) for clinical characterization of their malaria episodes, and accurate diagnosis of concomitant conditions. PCR diagnosis was performed in all patients to confirm *Plasmodium* species and exclude coinfections with *P. falciparum*. During a nine-month period, 154 patients were admitted at our hospital with an associated diagnosis of *P. vivax* infection. Of these, 32 were children younger than 12 years of age and 81 fulfilled one or more of the WHO severity criteria defined for *P. falciparum*. Previous co-morbidities were present in 82% of these inpatients. The most common severe criterion was hyperbilirrubinemia (bilirubin> 3.0 mg/dL), occurring in 77% of the patients (62/81), followed by severe anemia (Hemoglobin < 7.0 g/dL in adults and < 5.0 in children), which was present in 30% of the patients (25/81). Other complications that occurred include respiratory distress, acute kidney failure and splenic rupture. G6PD deficiency was diagnosed in 26 patients, who presented with hemolytic anemia following use of primaquine. Two patients died during follow-up, one of them with extensive subdural hematoma (a 76-year-old patient with previous arterial hypertension) and the other one, a patient with chronic liver failure, due to severe gastrointestinal bleeding. *P. vivax* infection may present with a wide-array of clinical complications both in children and adults. The use of primaquine for radical cure of *P. vivax* hypnozoites increases the risk of hemolytic complications among people with G6PD deficiency. More detailed analysis and case control studies being undertaken at our centre will certainly help to identify risk factors for complications associated with *P. vivax* infection, and validate WHO definitions currently based only on *P. falciparum* cases.

142

THE EFFECT OF CHANGES IN RAINFALL ON THE BURDEN OF MALARIA IN AREAS OF HIGH AND LOW TRANSMISSION SETTINGS

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The effect of rainfall on malaria risk may vary across differing transmission and environmental settings and further by the level of intervention deployment. Clarifying this relationship may be informative to malaria control programs. The objective of this study was to determine the effects of change in rainfall on the malaria burden in 2 different endemic settings. The Uganda Malaria Surveillance Project collects daily malaria morbidity data from 6 sentinel health facilities around Uganda. In this ongoing study, we utilized malaria data from the lowest and highest transmission sentinel settings: Kamwezi Health Center in Kabale district (EIR < 1) and Aduku Health Centre, in Apac district (EIR = 1564) respectively. Routinely collected daily rainfall data from Kabale and Apac districts were obtained from Uganda's national meteorological department. We used linear regression models to assess the association between total monthly rainfall and malaria slide positivity rate (SPR) for the next month, adjusting for age and number of malaria laboratory tests done. This preliminary analysis includes data collected over 26 months in Kabale/Kamwezi and 41 months in Apac/Aduku. The median total monthly rainfall in Kabale was 92.7mm (IQR 61-114.6) and 112.3mm (IQR 4.4-202.1) in Aduku. Age-standardized SPRs ranged from 13.4% to 65.6% in Kamwezi (median 29.4%) and from 26.2%% to 57.7% in Aduku (median 46.1%). In Kabale, a 1mm increase in rainfall increased the SPR by 0.002% (p = 0.036). Changes in rainfall were not associated with changes in malaria diagnosed in Apac. These initial findings suggest a modest association between increase in rainfall and subsequent malaria upsurges in areas of low but not high transmission intensity. Data collection on intervention coverage and environmental factors is ongoing.

143

RISK FACTORS FOR ANAEMIA IN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA IN THE MOUNT CAMEROON REGION: ROLES OF NUTRITION, WORMWOOD AND IRON DEFIECIENCY

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The lack of up to date epidemiological data on malaria and anaemia in many parts of Cameroon is a serious handicap towards effective control of these conditions. This study had as objectives to identify the risk factors for anaemia and to determine the influence of wormwood, nutritional status and iron deficiency on malaria anaemia. Malariometric and nutritional indices were measured in 817 children between the ages of 6 months to 14 years over a period of 2 years. The prevalence of asexual parasitaemia was 75.0% (613). The overall prevalence of anaemia (Hb < 11g/dl) was 81.3% (664). The prevalence of pyrexia was 24.7% with a significant positive correlation between temperature and malaria parasitaemia density (r = 0.03, P = 0.01). Splenomegaly was significantly prevalent (P < 0.01) in gametocyte positive children (36.2%, 38/105) when compared with gametocyte negative children 19.3%. The prevalence of splenomegaly (23.6%) and gametocytaemia (25.6%) confirms malaria endemicity in this region. The prevalence of gametocytaemia may be due to the observed low parasitaemia and the high prevalence of anaemia. Malnutrition as assessed by a < -2 z-score in any one of the anthropometric indices height-for-age (HA), weight-for-age (WA), weight-for-height (WH), was prevalent in 22.2% of the children. The prevalence of stunting (19.6%) was more common than underweight (7.2%) and wasting (2.2%) which likely reflects the low socio economic status of the inhabitants. Multilinear regression analysis showed the level of education of caregiver (P < 0.05), high WBC count (P < 0.0001), parasitaemia density (P <0.01), length of fever > 2 days (P < 0.01), spleen size (P < 0.05), male sex (P < 0.05), management of onset of malaria by the caregiver (P <0.005), stunting (P < 0.05), ferritin and transferrin (P < 0.001) were the risk factors for anaemia. Iron deficiency had a significant influence on malarial anaemia although a large proportion of anaemia cases could not be explained by iron deficiency indicating that malaria is a significant cause of anaemia in the study population

144

IMPLEMENTING THE FIRST NATIONAL SCHOOL MALARIA SURVEY IN KENYA: PROCESS, MAIN FINDINGS AND IMPLICATIONS FOR SURVEILLANCE AND CONTROL

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National malaria control programmes require up-to-date, sub-national information on malaria transmission in order to guide intervention strategies according to malaria risk. In 2009, the Government of Kenya launched its second National Malaria Strategy for the period 2009-2017 which includes a new Malaria-free Schools Initiative. Here we present results from a national survey of malaria infection and coverage of malaria control interventions among Kenyan 55,737 children in 552 schools. Infection was defined in the field using malaria rapid diagnostic tests (RDT) and haemoglobin assessed using a portable photometer. All RDT-positives and a selection of RDT-negatives were validated through microscopy. A questionnaire was administered to pupils to obtain data on mosquito net ownership and use and when treated, recent travel history, recent history of illness, and socio-economic and household variables. The overall prevalence of *Plasmodium* infection and anaemia was 7.2% and 20.8% respectively. 22.4% of children reported using an insecticide treated net (ITN). Patterns of infection, anaemia and net use varied markedly across the country, with infection prevalence being highest in western Kenya. Malaria risk in the western highlands and along the Kenyan coast was more geographically heterogeneous, whereas there was extremely low malaria risk in central Kenya. Only 1.7% of schools reported ITN use >60%. These data show that there are large areas of Kenya, mainly in Central, Eastern and Rift Valley provinces, that do not merit any direct school-based malaria intervention. School-based interventions, coupled with strengthened community-based strategies, are warranted in western Kenya, whereas a geographic targeting of intervention suites should be considered in the western highlands and along the Kenyan coast. School malaria surveys provide a rapid, cheap and sustainable approach to malaria surveillance and risk mapping and should be seen as an essential component of future monitoring and evaluation strategies in Kenya.

145

MALARIA IN NAIROBI

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Research on the epidemiology of malaria in Africa has traditionally focused on areas of stable, high transmission where infection results in high disease burden. Consequently, little is known about its epidemiology in low transmission settings such as urban areas. Despite documented transmission in the area currently occupied by Nairobi pre-1940 there have only been sporadic studies of malaria risk in recent years. A more detailed examination of the epidemiology of host infection risks is necessary to develop specific treatment and prevention guidelines for Nairobi residents. Four different approaches will be used to address the data deficiencies. First, clinic fever surveys which involve a rapid assessment of the prevalence of malaria infection among patients presenting to 10 clinics with a history of fever. Second, school surveys where children were examined for malaria infection using RDTs. These same 10 schools will be revisited and an additional filter paper blood spot will be collected for PCR and serological analysis. Third, rapid assessment of the quality of care, diagnosis of malaria and case management in health facilities. And finally surveys on EPI attendees where we aim to identify the most plausible estimation of autochthonous transmission by examining the prevalence of infection and history of exposure among resident children. In March 2009 1333 children were examined, 5.5% were identified as having a positive RDT. Among the 74 positive cases 40.5% had travelled outside of Nairobi in the last eight weeks and of these 70% had travelled to an area classified as malaria-risk. However a similar proportion of test negatives had travelled in the last eight weeks 556 (44.5%) and of these 32.6% had travelled to a malaria risk district. In July 926 children were examined. Blood slides were re-examined by expert microscopists for all 17 RDT positives and 10% of RDT negatives. 1.84% of the children had a positive RDT result but this number dropped to 1.08% when the results were confirmed via microscopy and only one child has a history of travel. At this stage the possibility of autochthonous transmission cannot be ruled out and additional results can inform on the true risks.

146

MALARIA HYPO-ENDEMIC FOCI AND EPIDEMIC RISK ON JAVA AND BALI, INDONESIA IN 2009

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Recent calls for strategies aimed at malaria elimination emphasize the importance of prompt identification of persistent or erupting foci of transmission. The challenge is to place limited resources precisely where and when needed. Two essential measures of malaria intensity in elimination stages are the malaria basic reproductive number (Ro) and the reproductive number under some level of control (Rc). Bayesian geostatistical models were used to predict continuous maps of *Plasmodium falciparum* parasite rate (*Pf*PR) from over 200 spatially independent parasite rate estimates from community surveys conducted in Java and Bali in the Indonesian archipelago. Subsequently, stochastics models were employed to define the relationship between *Pf*PR, entomological innoculation rate (EIR) for *P. falciparum* (*Pf*EIR) and the basic reproductive rate for *P. falciparum* (*Pf*Ro). A transmission intensity map for *P. falciparum* (*Pf*Rc) was then generated from the models. The higher spatial resolution *Pf*Rc map is expected to provide a rational basis for malaria elimination planning and setting precise targets of intervention. These results are summarized across Java and Bali and the implications for elimination elaborated.

147

CLINICAL LABORATORY REFERENCE RANGES DERIVED FROM RURAL HEALTHY LOCAL POPULATION OF HEALTH DISTRICT OF SAPONÉ IN BURKINA FASO

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In Africa, biological parameters that are used in clinical laboratories are from Europe and the United States. The conduct of clinical trials in African sites using references values from others population excludes potential volunteers and makes adverse events assessment challenging as the primary objective of Phase I trials is to demonstrate the safety of the investigational according to CFR and ICH guidelines. For the planning of malaria vaccine trials in Saponé Health District, a malaria endemic area in Burkina Faso, we conducted a study to establish the biological reference ranges for biochemistry, hematology among healthy local population. Two cross sectional surveys conducted respectively during malaria high and low transmission season in healthy adults and children from 14 randomly selected villages out of 89 villages of Sapone Health District and stratified in equal proportions in 7 age-groups and gender for adults: 6 months- 1 year, 1-3 years, 3-6 years, 6-10 years and 10-15 years, 15-45 years male, 15-45 years female. The hematology and chemistry analysis were done with validated analyzer with strong records of internal and external quality controls. Data from the two surveys were pooled and using the methods described in the NCCLS-approved guideline, reference intervals for each measured parameter were calculated non-parametrically by taking the 2.5 and 97.5 percentiles of the observed samples values. From a total of 2520 patients who were screened during both surveys, 2049 were included in the analysis with at least 270 volunteers per age-group and gender for adults. Estimated Hemoglobin and hematocrit references range were lower in our local population than the western ones while alkaline phosphatase, ALT, AST, WBC, lymphocytes were higher in the former. Similar references intervals were found with electrolytes (Na, K, Ca, Cl), creatinin, RBC, total bilirubin, direct bilirubin, albumin and glucose. In conclusion, reference intervals of haematological and biochemical indices based on results from population of developed countries of the same age are different to the estimated values for population of the Health District of Saponé in Burkina Faso. These findings support implementation of malaria vaccines trials in this area using site-specific biological references intervals for enrolment and monitoring of patients.

148

SPONTANEOUS CLEARANCE OF *PLASMODIUM FALCIPARUM* PARASITEMIA WAS MORE COMMON IN UGANDAN CHILDREN WITH SICKLE CELL TRAIT THAN IN THOSE WITH NORMAL HEMOGLOBIN

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The genetic abnormalities glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and sickle cell trait (HbAS) offer protection against *falciparum* malaria. They may protect through improved immune clearance of parasites, but this hypothesis has not been tested clinically. 601 randomly selected children from Kampala, aged 1-10, were followed for a median of 1.4 years. Blood smears were read every 30 days and any time a child presented with fever or history of fever. Children with malaria, defined as asexual parasites on blood smear and fever, were treated after randomization to one of 3 combination therapy regimens. Hemoglobin electrophoresis for HbAS and spectrophotometry to assess G-6-PD activity (deficient: <110 mU/109 erythrocytes) were performed at enrollment. HbSS children were excluded. To follow individual strains, parasitemic samples were genotyped by assessment of polymorphisms in *merozoite surface protein 2* by nested PCR and capillary electrophoresis. Our primary outcome was spontaneous clearance of parasites, a surrogate for effective antimalarial immunity. With HbAS and G-6-PD deficiency as our predictor variables, we used generalized estimating equations to estimate the relative risk of spontaneous clearance of parasites, adjusting for age. Ninety-nine children (16.5%) had HbAS and 62 (10.3%) were G-6-PD deficient. Genotyping revealed 2295 parasite strains in 370 subjects, giving an incidence of parasitemia of 2.8 per person year. Older children were more likely to clear infections once parasitemic (RR = 1.16 / year of age, 95%CI 1.10-1.23, p<0.001). Children with HbAS were significantly more likely to clear infections than those with HbAA (RR = 1.43, 95% CI 1.01-2.01, p=0.04). No association was found between G-6-PD deficiency and rate of clearance of infections. These results support the hypothesis that HbAS is protective against *falciparum* malaria, at least in part, due to increased immune clearance of parasites.

149

SPATIAL ANALYSIS OF PEDIATRIC MALARIA IN WESTERN KENYA

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There is data to suggest that malaria severity is influenced by the force of parasite exposure. The latter is susceptible to a variety of spatial and temporal variables that affect mosquito dynamics. Geographical information systems (GIS) provide an easier way of correlating disease to spatial data. In the present study, spatial information on children presenting at Kisumu District Hospital (KDH), Western Kenya, with either severe or uncomplicated malaria were mapped in relation to rainfall, drainage, altitude and land use. Geographic identifier of patient homes (nearest market center, nearest schools and proximity to known unique features) were collected from 120 subjects attending KDH with either severe malaria (N=60) or mild malaria. Points representing the geographic location of the cases were overlaid onto a map of drainage features (rivers, lake and flood plains), rainfall (800-2000 mM) and topography. Of the spatial features, only rainfall and drainage affected distribution of malaria cases. 93% of patients fell within a distance of 2 km from the rivers basins, lake and flood plains but there was no particular clustering of malaria in relation to disease severity. All the patients fell within the 800-2000 mM annual rainfall belt, with 14% falling in the 800-1200 mM, 55.6% in the 1200-1600 mM and 23.6% within the 1600-2000 mM. In conclusion, drainage and rainfall are the major determinants of exposure to malaria in the holoendemic Lake Victoria basin. Data will be presented to show how GIS can be utilized to describe determinants of malaria exposure and how to achieve targeted interruption of transmission.

150

HUMAN MOVEMENT PATTERNS RELEVANT FOR MALARIA TRANSMISSION IN TANZANIA AND MALI

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Human movement patterns are important drivers of disease transmission, and yet there are few studies which quantify these patterns across large areas, and very few in rural Africa. The demographic and health surveys (DHS) include questions on overnight trips in the previous 12 months, which are asked to adults aged 15-45 of both sexes. These data allow within and between country comparisons of travel patterns relevant for malaria transmission. Here, we compare travel patterns in Tanzania and Mali, as a first direct comparison between east and west Africa. Data on the number of visits in the last 12 months for more than 30,000 adults were analysed using a generalised logit model. Probabilities of making no visits, 1 visit, 2-5 visits and more than 5 visits in the last 12 months were calculated with reference to gender, region of residence, age, occupation and wealth index. In both Tanzania and Mali, all these variables were found to be important predictors of frequency of travel, (p<0.001). Men had higher odds of travelling than women. For example, the odds of travelling more than 5 times (as opposed to 0,1 or 2-5 times) for men are 5.385 (95% CI 4.521-6.415) times those for women in Tanzania (9.917 (95% CI 8.269-11.893) for Mali). Those with a low wealth index were less likely to travel for an overnight visit. For example, those with the lowest income have an odds of 0.487 (95%CI 0.342-0.695) that of the highest wealth class of travelling at least once for Mali when compared with the odds of not travelling. Regions with higher population densities were associated with a lower probability of making no overnight visits. The probability of making at least one visit was significantly different between the two countries, even when accounting for other factors (p<0.0001). Overall 41% of individuals made at least one overnight visit in Tanzania, whereas 32% of those surveyed in Mali made at least one overnight visit. These results show that whilst there are shared determinants of travel patterns, there are important differences in national characteristics. Further study is required to disentangle the reasons for these differences and their consequences for malaria transmission.

151

SITUATION ANALYSIS AND PUBLIC HEALTH INTERVENTIONS TO PREVENT MALARIA IN KENYA

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In Kenya malaria is found in nearly all its provinces except in Nairobi. The leading provinces in malaria endemicity are those near the Indian Ocean and the south and lakes Victoria in western province and Lake Turkana in the north. Nairobi is a focus of our study due to its being the capital but also it is the melting point of the all country, every of Kenya's fourty two communities are found in Nairobi. The site of the study was the Mary Immaculate Clinic in Nairobi. The Clinic provides health care for the urban poor with a population of more than 100,000 inhabitants of Nairobi especially in the informal settlements. We counted the number of positive and negative patients who were tested for malaria since the year 2005 to 2009, a span of five years. The criteria used to according to the clinic protocol for malaria testing is, fever with a high temperature of 38C, joint pains, chills, among others. Testing was done by microscopy. A total of 8157 patients were counted from the laboratory records book. Many

of the patients that were tested have only basic primary education. The malaria species endemic was *Plasmodium falciparum*. In the year 2005 the total number of malaria positive was 17%. A total of 83% patients were negative. The total of positive and negatives were 1860. In the year 2006 the total malaria positives were 242 (16%) out of 1509 (84%), in these two years there was no significant increase in malaria patients with time. In the year 2007 there were 217 (15%) malaria positives and 1488 (85%) malaria negatives. There were 200 (12%) malaria positives in 2008 and 1653 (88%) negative. Despite the increase in the number of patients tested in 2008 there were no significant increase in positivity in those two years. The same can be concluded of the year 2009. In conclusion, the malaria data in the five years confirms that the occurrence of malaria in Nairobi is low. Though only 1218(15%) out of 8157 (85%) had malaria, the positives are clinically significant, but cannot be referred to qualify Nairobi as a malaria zone. It is therefore prudent to for the healthcare system to take more action to educate the public when travelling to use preventive measures and adhere to treatment when sick with malaria.

152

INDIVIDUAL HETEROGENEITY AND THE POTENTIAL REBOUND EFFECT OF MALARIA INTERVENTION STRATEGIES

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Most trials designed to test the effectiveness of a malaria prevention method, such as insecticide-treated nets (ITNs) or insecticide-treated curtains (ITCs), randomize newborns or clusters of newborns to one or more treatment groups. These trials are then analyzed following the intention-to-treat framework. It is hypothesized that infants using malaria prevention methods early in life do not have the opportunity to build adequate immunity to malaria, thus causing higher mortality rates in these infants later in life. This is often referred to as a "rebound" in mortality. We focus on the situation in which the all-cause mortality rates of two groups of randomized infants (e.g., ITNs and no nets) are of interest. We assume that ITNs are given to the group with no nets at the end of year one and that all children, including those initially assigned to the ITN group, are followed for another year. Mortality rates for the two groups are compared for year one and again for year two to assess the treatment effectiveness. If a rebound effect exists, then the children who were initially randomized to the ITN group should have a higher mortality rate in the second year of life than the children initially randomized to the non-ITN group. Biological variation between individuals can account for a large portion of the variability seen in medical and public health studies and can distort observed effects (Aalen, 1998). We demonstrate with randomly generated data that a potential rebound effect can be caused by individual heterogeneity, as treatment groups followed from the end of year one are no longer randomized. We also use data from a randomized, controlled trial conducted in Burkina Faso (Diallo et al., 2004) to illustrate the relationship between individual heterogeneity and the rebound effect. This study found a mortality rate ratio of 1.16 in children aged 24-59 months when comparing original treatment groups after all study participants had been allocated ITCs, and we show that this effect could have arisen from individual heterogeneity alone.

153

SIMULATING MALARIA TRANSMISSION DYNAMICS IN THE PILOT SITES OF THE COLOMBIAN INTEGRATED NATIONAL ADAPTATION PLAN: STEPS FORWARD OF THE INTEGRATED SURVEILLANCE AND CONTROL SYSTEM

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Changes in climatic conditions are likely to alter malaria incidence and spatial distribution in Colombia. As part of the Integrated National Adaptation Plan, the Colombian Institute of Health is working on the implementation of a proactive, collaborative, multidisciplinary, integrated surveillance and control system (ISCS). The aim of this initiative is to improve risk assessments of malaria transmission in order to facilitate effective allocation of health resources and more cost-effective preventive responses. One of its key components is an Early Warning System Framework, in which we are proposing several dynamical and statistical models. Dynamical models, in particular, are being used to integrate climatic variables with non-climatic factors in order to simulate malaria transmission dynamics. Twelve process-based models were studied and included in a single multi-model ensemble. Five tools were initially applied in the pilot sites where the ISCS is being implemented. Activities included the characterization of local eco-epidemiological settings and numerical simulations. Characteristics such as general profile (population at risk, natural resources, economic activities), climatic conditions (climatology, long-term trends), entomology (primary and secondary vectors, breeding sites, feeding frequencies, preferences), malaria situation (annual cycles of malaria incidence, stability conditions), and non-climatic factors (including control campaigns) were analyzed to assess local conditions. Simulations included retrospective experiments (base scenarios, changes in initial conditions, local settings, sensitivity analyses, and uncertainties) of at least 8-year simulation periods, as well as short-, medium- and long-term future changing scenarios. Complementary activities included the study of local spatial patterns of vectorial capacity, descriptions of the vulnerability of populations at risk, and a conceptual framework for the analysis of non-climatic drivers. Outreach activities included the design of interactive and online platforms as well as the documentation of our experiences. Dynamical models have improved our understanding of malaria complexity, allowed us to estimate previous malaria outbreaks in the selected pilot sites, and helped us to investigate decision-making processes. All these activities constitute steps forward in the implementation of the Colombian ISCS.

154

MALARIA ASSOCIATED SYMPTOMS IN PREGNANT WOMEN: RESULTS OF A COHORT FOLLOW-UP IN BENIN

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Little is known on the symptoms of malaria infected pregnant women in stable endemic areas, as it is generally admitted they have acquired an immunity protecting them from acute clinical signs. By following-up

Beninese pregnant women, this study aims to evaluate the clinical burden of malaria in a highly endemic area. An ongoing prospective cohort of 1039 women followed monthly from their first antenatal visit (ANV) until delivery is conducted in three rural dispensaries since August 2008 in Benin. 570 women seen at ANVs, unscheduled visits and at delivery were analysed for the presence of symptoms. We used a multivariate logistic regression to determine the association between symptoms and malaria infection assessed by a positive rapid diagnostic test (RDT). During routine ANVs, headache was the only symptom associated with a higher risk of malaria (aOR=2.6; p<0.001) and was reported by 35% of infected women. On the occasion of unscheduled visits, fever (aOR= 4.1; p<0.001), headache (aOR= 2.1; p=0.01) and shivering (aOR= 3.2; p<0.001) were significantly associated with a malaria infection and 82% of infected women presented at least one of these symptoms. We found an increasing proportion of positive RDTs in late pregnancy more than one month after the last intermittent preventive treatment dose (IPTp); moreover malaria infections during unscheduled visits occurred long after the last IPTp intake. In conclusion, the majority of pregnant women were symptomless during routine visits when infected with malaria in an endemic stable area. Only, during unscheduled visits a significant proportion of infected women were symptomatic. The prevention of malaria in pregnancy could be improved by using systematic RDTs to identify infected women consulting during non routine visits. The design of IPTp could also be optimized by reassessing the number of doses and time of administration of SP.

155

NATIONWIDE PREVALENCE OF MALARIA IN CAMBODIA IN 2007: COMPARISON OF MICROSCOPY AND PCR

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In order to assess the current status of malaria in Cambodia and to compare it with the situation found in 2004, a nationwide malaria survey was conducted in November-December 2007, at the end of the rainy season, the time of peak malaria transmission. This was a stratified, multi-stage, cluster sampling survey. The country was divided into three domains based on expected malaria prevalence. The domain that included the provinces immediately around Phnom Penh was not surveyed, due to the very low prevalence found in previous surveys. The remaining provinces were divided into domains 1 and 2. Within each domain 38 clusters (villages) were selected; the clusters were stratified according to risk zones based on the distance from the village to the nearest forest (<250 m, 251-1000 m, 1-2 km, 2-5 km). Within each cluster 40 households were sampled, and from each household, 4 individuals provided malaria smears and filter paper blood spots for PCR-based diagnosis using the mitochondrial cytochrome b gene as a target. Based on microscopy, the overall estimated malaria prevalence and prevalences of *P. falciparum* and *P. vivax* infection in the sampled domains were 2.9% (95% CI, 1.8-4.6%), 1.6% (0.9-2.7%), and 0.9% (0.6-1.6%) respectively. The corresponding prevalences found in 2004 were 4.4% (2.8-6.8%), 2.9% (1.7-5.1%), and 1.3% (0.8-2.1%); this decline in prevalence, while appreciable, was not statistically significant. In order to determine the extent to which microscopy might underestimate the malaria prevalence, we performed PCR on 7707 samples; in these samples the malaria prevalences estimated by microscopy and PCR were 2.8% and 6.9%, respectively; 289 of 7162 microscopy negative samples (4.0%) were positive by PCR. The high prevalence of infection undetected by microscopy suggests that prevalence surveys based only on microscopy may significantly underestimate malaria prevalence. If these sub-microscopic infections contribute to transmission, then mass screening and treatment based on microscopy alone may miss a significant reservoir of infection.

156

SPATIO-TEMPORAL DISTRIBUTION OF MALARIA IN HAINAN PROVINCE, CHINA

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Hainan Province is one of the regions of the highest malaria incidence in China. Our study analyzed the distribution of malaria and the change of main epidemic areas from 1995 to 2008, to provide basis for the prevention and control of malaria in Hainan Province. The study was based on the data of each county/city between 1995 and 2008. Records of malaria cases were obtained from Hainan Center for Disease Control and Prevention and demographic data from Hainan statistical yearbook. Cluster analysis of time-space scanning was performed with the maximum cluster size of 25% of the population used SatScan 8.0. The temporal cluster analysis of 1995-2008 showed that 2003-2004 was the most likely cluster (*RR*=1.86, *P*=0.001). The space-time cluster analysis of 1995-2008 showed 7 counties/cities (San Ya, Bao Ting, Le Dong, Wu Zhishan, Ling Shui, Bai Sha and Qiong Zhong) in 2003-2004 was the most likely cluster (*Incidence*=2671.0/100,000, *RR*=4.97, *P*=0.001). The space-time cluster analysis of 1995-2002 showed 5 counties/cities (Bao Ting, San Ya, Wu Zhishan, Ling Shui and Qiong Zhong) in 1997-1998 was the most likely cluster (*Incidence*=1852.8/100,000, *RR*=4.49, *P*=0.001) and 3 counties/cities (Chang Jiang, Dong Fang and Bai Sha) in 2001-2002 the secondary one (*Incidence*=1258.6/100,000, *RR*=3.25, *P*=0.001). The space-time cluster analysis of 2005-2008 showed 5 counties/cities (Ling Shui, Bao Ting, Wan Ning, Qiong Zhong and Wu Zhishan) in 2005 was the most likely cluster (*Incidence*=1193.7/100,000, *RR*=4.55, *P*=0.001) and 4 counties/cities (Dong Fang, Chang Jiang Le Dong and Bai Sha) in 2006 the secondary one (*Incidence*=1038.9/100,000, *RR*=3.30, *P*=0.001). In conclusion, during 1995-2008, malaria incidence reached its peak in 2003-2004 and the southern Hainan Province was the main epidemic area. Although the average incidence decreased, the main epidemic area was expanded to the southeastern and southwestern Hainan Province gradually. Hence, future public health planning and resource allocation in Hainan Province should be focused on these areas.

157

INSIGHT INTO ANTIGENIC DIVERSITY OF VAR2CSA-DBL5ε DOMAIN FROM MULTIPLE *PLASMODIUM FALCIPARUM* PLACENTAL ISOLATES

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High levels of anti-VAR2CSA antibodies levels are associated with protection against pregnancy-associated malaria. VAR2CSA contains molecular signatures associated with parity in one of its domain, and variants preferentially infecting primigravidae are thought to be the most virulent. Therefore it is critical to identify sequence characteristics of this molecule that can interfere with immune response. Highly conserved domains of VAR2CSA such as DBL5ε are likely to contain conserved epitopes, and therefore constitute attractive targets for vaccine development. Sequences of the VAR2CSA-DBL5ε domain obtained from cDNA of 40 placental isolates were analysed by experimental and in silico tools. Competition ELISA assays on two DBL5ε variants, using women plasma samples from two different areas and mice specific antisera, indicated that DBL5ε possess conserved and cross-reactive B cell epitopes. Peptide ELISA identified conserved areas that are recognised by naturally acquired antibodies. Specific antibodies against these peptides

labelled the native proteins on the surface of placental parasites. Despite high sequence homology, both VAR2CSA DBL5ε recombinant proteins displayed different recognition patterns by plasma from malaria-exposed women, and their ability to bind proteoglycans. Sequence analyses showed that, like the previously characterised VAR2CSA DBL3X domain, DBL5ε also contains motifs that discriminate parasites according to donor's parity. In conclusion, this study provides insights into conserved and exposed B cell epitopes in DBL5ε that can act as potential mediator for cross reactivity. The importance of sequence variation in VAR2CSA as a critical challenge for vaccine development is highlighted. As the final conformation of the entire VAR2CSA molecule seems to be essential to its functionality, identification of sequence variation sites in distinct locations within VAR2CSA that affect its antigenic and/or binding properties is of major interest in the effort of developing an efficient VAR2CSA-based vaccine. Motifs associated to parasite segregation according to parity are among these critical issues.

158

SITE CHARACTERIZATION FOR A MALARIA VACCINE TRIAL IN THE SAPONÉ HEALTH DISTRICT IN BURKINA FASO: SEASONAL PREVALENCE OF MAIN PARASITES INFESTATION

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Populations living in areas where future malaria vaccine trials may be conducted must be characterized not only with respect to the parameters that will be used to establish safety, but also with respect to conditions that may modify the immune response to candidate malaria vaccines. Studies conducted in Africa and Asia indicates that helminths can influence the acquisition of immunity against *Plasmodium* by driving the immune responses towards the production of the non-cytophilic subclasses. The aim of this study was to estimate the prevalence of various parasitic infections according to season in potential participants to malaria candidate vaccine trials in Burkina Faso. We conducted 2 community-based cross sectional surveys in volunteers aged 2 to 45 years in the Sapone health district. Survey 1 was performed during the rainy season, and the second at the dry season. During each survey, clinical examination has been performed and blood samples have been taken for malaria and *Wuchereria bancrofti* diagnosis. Stools and urine were also collected for determination of helminthes and *Schistosoma hematobium*. The diagnosis of intestinal helminthes was done by Kato-Katz thick smear examination technique. The mean age of the volunteers was similar during the 2 surveys (p=0.44). From 1587 stools samples analyzed, 132 (8.3%) had helminth or other intestinal infections. The prevalences were higher at the rainy season as compared to the dry season. The main helminth infections were *Ankylostoma duodenale* (5.9% vs 2.1%; P<0.00), *Ascaris lumbricoides* (1.7% vs 0%; P<0.00), *Trichuris trichiuria* (0.8% vs 0%; P<0.00). Others intestinal parasites were *Hymenolepis nana* and *Taenia* sp. (4.5% vs 2.1%; P<0.00). The seroprevalence of *W. bancrofti* was 11.0% (12.8% vs 9.4%, P=0.03). *S. hematobium* infection was present in 2.3% (1.7% vs 2.9%, P=0.13) of the study population. According to age group the prevalence of malaria infection was higher at the rainy season (< 5years: 68.9%; ≥ 5years: 54.8%) as compared to the dry season (< 5years: 57.8%; ≥ 5years: 46.3%). In conclusion, these data show diversity and intensity of parasitic infections in Saponé health district area according to malaria transmission season. The trends of helminths infections and malaria infection coincide and are both high during the malaria high transmission season. This should be considered when designing future malaria vaccine trial.

159

IMMUNOLOGICAL EFFICACY OF VACCINE-INDUCED ANTIGEN-SPECIFIC CD8+ T CELLS AGAINST *PLASMODIUM YOELII* BLOOD STAGE INFECTION

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There is a consensus that CD8+ T cells are critical for conferring hosts' protective immunity against the malarial liver stage; however on the contrary, the lack of MHC molecule on red blood cells has questioned their protective roles against its blood stage infection. This skepticism was supported by an observation that the depletion of CD8+ T cells during the malarial blood stage infection did not affect its natural course and outcome. However, since the CD8+ T cell-mediated vaccine strategy has presented new development in recent years, it is worth elucidating the immunological efficacy of the active induction of antigen-specific CD8+ T cells, particularly the prime-boost vaccination strategy which is the most effective vaccination protocol for the induction of maximal number of antigen-specific CD8+ T cells. To address the question whether the actively induced CD8+ T cells in maximal number are capable for conferring hosts' protective immunity against the malarial blood stage, we have established an experimental system by generating a genetically engineered *Plasmodium yoelii* which expresses a *Trypanosoma cruzi* antigen-derived, H-2Kb-restricted-CD8+ T cell epitope, ANYNFTLV. Expression of the epitope by the transgenic parasites was confirmed by the detection of ANYNFTLV-specific CD8+ T cells in mice, either which were immunized with adjuvant-emulsified parasitized red blood cells or which were cured by the injection of chloroquine after the infection with transgenic parasites. We have then tested the immunological efficacy of the prime-boost recombinant virus vector vaccination, the multiple passive transfers of ANYNFTLV-specific CD8+ T cell line and the combination of both against the challenge infection with the ANYNFTLV-expressing transgenic parasites. The critical roles of CD8+ T cells during the malarial blood stage infection and their background immunological mechanisms will be presented and discussed.

160

HETEROLOGOUS PRIME-BOOST VACCINATION WITH ADCH63 AND MVA EXPRESSING MSP1 CAN INDUCE PROTECTIVE EFFICACY AGAINST SPOROZOITE CHALLENGE IN VOLUNTEERS

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Viral vectored vaccines encoding blood-stage malaria antigens can stimulate potent cellular and humoral immune responses in mice and rhesus macaques and induce protective efficacy in rodent malaria models. We sought to test the safety, immunogenicity and efficacy of this approach in a Phase I/IIa clinical trial using the simian adenovirus 63 (AdCh63) and the poxvirus MVA encoding a novel insert including conserved blocks of sequence and both alleles of the 42kDa C-terminus of the blood-stage malaria antigen MSP1. In a Phase I dose escalation

study in Oxford, UK, 16 healthy malaria naive volunteers were primed with AdCh63 MSP1 and 12 of these volunteers boosted 8 weeks later with MVA MSP1. High level antibody responses were measured against the C-terminal 19kDa region of MSP1 ($MSP1_{19}$) as well as the strongest T cells responses yet reported by subunit vaccination, as measured by ex-vivo IFN-γ ELIspot using peptides spanning the entire vaccine antigen. Given the qualitatively different type of immune responses induced by viral vector vaccines (in comparison to recombinant protein-in-adjuvant vaccines routinely used by other researchers), three vaccinees and six unvaccinated controls underwent sporozoite challenge three weeks after the MVA boost as a Phase IIa safety study. These vaccinees demonstrated a significant delay in time to diagnosis (by positive blood film) compared with the unvaccinated controls (P=0.032). No unexpected adverse events were observed. This is the first demonstration of statistically significant clinical efficacy induced by a vaccine targeting the blood-stage antigen MSP1, and provides the first evidence that vaccines inducing cell mediated responses in conjunction with antibody responses to a blood-stage antigen used alone are safe as well as effective. This AdCh63-MVA viral vectored vaccine regimen also provides a new and safe approach for the development of vaccines for other infectious diseases where it is likely that strong cellular and humoral immunity will be required for protective efficacy in humans.

161

EXTENDED SAFETY, IMMUNOGENICITY AND EFFICACY OF WALTER REED ARMY INSTITUTE OF RESEARCH'S AMA-1 MALARIA VACCINE (FMP2.1) ADJUVANTED IN GSK BIOLOGICALS' AS02A IN 1-6 YEAR OLD CHILDREN IN BANDIAGARA, MALI

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The malaria vaccine candidate FMP2.1/AS02A was recently evaluated in Bandiagara, Mali, West Africa. Data collected up to 6 months following the final immunization showed acceptable safety, and high-level antibody responses, but limited efficacy (VE) against first clinical malaria episodes (VE 17.4%, p=0.175) or against any clinical episode (VE 20.0%, p=0.068). Extended safety, immunogenicity and efficacy data were collected for 24 months. Four hundred healthy children aged 1-6 were randomized 1:1 to receive three doses of 50 µg of FMP2.1 in 0.5mL of AS02A or rabies vaccine, 30 days apart. The primary efficacy endpoint is time to first or only clinical malaria episode occurring between randomization and six months after the third immunization. Secondary endpoints include time to first clinical malaria episode and incidence of all clinical episodes (using increasing parasitemia thresholds) occurring during the entire follow-up period. The vaccine showed no safety signal, and was well-tolerated. High-level antibody responses were maintained and boosted during the subsequent malaria season. Extended efficacy of the vaccine against first clinical malaria episodes was 7.6% (p=0.507) and against any clinical episode was 9.9% (p=0.193). In conclusion, the lack of extended efficacy of the vaccine in the second malaria transmission season may be due to waning immunity that is not reflected in anti-AMA1 antibodies as measured by ELISA, or to a shift in AMA1 haplotypes at the site. Studies to determine the precise immune correlates of vaccine-induced immunity and detailed analyses of allele-specific efficacy and vaccine selection may lead to strategies to develop an improved AMA-1 vaccine.

162

CONSTRUCTION AND IMMUNOGENICITY OF A DNA VACCINE PLASMID ENCODING AMA-1 OF THE REEMERGING KOREAN *PLASMODIUM VIVAX*

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A DNA vaccine plasmid encoding *Plasmodium vivax* AMA-1 (PvAMA-1) of the reemerging *P. vivax* in Korea has been constructed and its immunogenicity examined in recipient BALB/c mice. A large AMA-1 protein band of about 56.8 kDa was obtained from COS7 cells transfected with the expression plasmid UBpcAMA-1. In BALB/c mice immunized intramuscularly 4 times with the PvAMA-1 vaccine with or without IL-12, serum IgG titers increased significantly compared to controls. Levels of IL-10, having a T-cell inhibitory function, were significantly depressed in immunized and immunized plus IL-12 treated mice. In contrast, IFN-γ levels showed little changes even in immunized plus IL-12 stimulated mice, and flow cytometry of spleen cells from immunized mice revealed no significant changes in the proportions of CD8+ cells and CD4+ cells. However, when mice were immunized using a gene gun, the proportion of CD8+ cells increased significantly in immunized and immunized plus IL-12 treated mice. The results indicate that the immunogenicity of the PvAMA-1 DNA vaccine was not strong enough when injected intramuscularly but suggest that the immunogenicity could be potentiated using the gene gun injection technique.

163

CHARACTERIZATION OF PFS25-EPA CONJUGATES BY AGAROSE GEL ELECTROPHORESIS AND WESTERN BLOTTING

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The Pfs25 protein is a transmission-blocking vaccine candidate against malaria. To enhance the immunogenicity, Pfs25 was conjugated to carrier proteins including a mutant, nontoxic *Pseudomonas aeruginosa* ExoProtein A (EPA) or the outer-membrane protein complex (OMPC) of *Neisseria meningitidis* serogroup B. The Pfs25 conjugates have been demonstrated to be 1000-fold greater in antibody titers when compared to the unconjugated Pfs25 in animal studies. In order to meet the requirements for clinical trials by regulatory agencies, the drug product identity and integrity have to be performed. However, due to the large size of molecular mass of the conjugates (greater than 700 kDa as estimated in this study), the most common characterization methods such as SDS-PAGE (suitable for proteins with molecular masses less than 200 kDa) and SDS-PAGE-based western blots are not suitable for quality control evaluations. The present study utilized the agarose as gel matrix and Pfs25-EPA conjugates as model protein samples for the integrity and identity studies. The Pfs25-EPA conjugates were analyzed by a 5 x 6 cm 2% Seakem ME (Lonza) agarose gel using tris-glycine running buffer conditions and

followed by Coomassie blue staining for visualization. For identity analysis by western blotting, the gels were subsequently transferred to PVDF membranes and probed by mAb 4B7 against Pfs25, mAb against penta-His tag, and polyclonal Ab against exotoxin A. Our results showed that the Pfs25-EPA conjugates had a range of molecular masses, approximately from 500 to 800 KDa as determined by the agarose gel. This molecular mass range was further confirmed by size-exclusion chromatography with multi-angle light scattering (SEC-MALS). Comparable protein conjugate migration patterns were detected by both agarose gel/Coomassie staining and agarose gel/western blotting. The results suggest that epitopes of Pfs25 and EPA remained detectable following the chemical modifications and electrophoresis using agarose gels. Overall, the present studies demonstrate that agarose gel alone or in combination with western blot analysis is a simple, reliable and economic technique to assess the identity and integrity of molecule with large molecular masses and will have a general application for analyzing proteins or their conjugates which are unable to be evaluated using SDS-PAGE.

164

PROTECTION AGAINST MALARIA CHALLENGE BY VACCINATION OF *AOTUS* MONKEYS WITH ADJUVANTED BLOOD STAGE MALARIA VACCINES

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The goal of this study is to evaluate a Blood Stage Antigen Combination (BSAC) containing leading vaccine candidates, AMA1, MSP1, MSP2, MSP3, and MSP4, in an *Aotus* challenge model. Previously we have demonstrated that AMA1 can confer in vivo protection of A. nancymai monkeys against Plasmodium *falciparum* challenge after monkeys received a recombinant AMA1 protein formulated with complete Freund's adjuvant, an adjuvant not suitable for human use. In order to down select a single human-compatible adjuvant system we first conducted an Aotus challenge study using recombinant AMA1 protein. Three groups (N=8) of A. nancymai monkeys received 3 doses of a recombinant AMA1 formulated with a synthetic TLR 4 agonist in an oil-in-water emulsion (EM005), an oil-in-water emulsion alone (EM001), or Alhydrogel+ the TLR9 agonist, CPG 10104. A control group received 3 doses of saline. Three weeks after the third vaccination monkeys were challenged with Aotus red blood cells infected with a homologous P. *falciparum* parasite. Thin blood films from individual monkeys were examined daily for detection of parasites in peripheral blood. Protection was assessed by the monkeys' abilities to control infections. While there were no statistically significant differences between the groups in protection from challenge, it appeared that similar limited levels of protection were observed in monkey groups receiving AMA1/Alhydrogel+CPG 10104 or AMA1/ EM005. In the second challenge study, the BSAC mixture was formulated with EM005. Three groups (N=11) of A. nancymai monkeys received 3 doses of i) an all-5 antigen mix formulated with EM005; ii) a 4-antigen mix (excluding AMA1) formulated with EM005; or iii) saline control. The monkeys were challenged with Aotus red blood cells infected with a homologous parasite, and protection was assessed by monkey's abilities to control infections. Significant protection was observed in both vaccine groups. Details of the study will be presented in the meeting.

165

DEVELOPMENT OF AN AD28-BASED, MULTIPLY-DELETED AND FIBER-MODIFIED, MULTI-ANTIGEN ADENOVIRAL-VECTORED VACCINE FOR MALARIA

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Malaria is the most burdensome parasitic disease of man, exacting an estimated toll of 863,000 deaths and 243 million clinical cases per year. Vaccines for malaria are greatly needed, but no technology is yet developed that completely addresses the current need. We are currently developing a capsid-modified, multivalent, adenoviral-vectored vaccine based on the adenoviral serotype 28 against *Plasmodium falciparum* for future human testing. The advantages of using adenoviral-vectored vaccines are several. They generate strong effector and helper T cell responses, strong antibody responses, and produce protective immunity in multiple disease models including against malaria in man. Previously, clinical tests of adenoviral-vectored vaccines for malaria have been based on adenoviral serotype 5. Ad5 has been shown to be safe in extensive clinical testing and produces strong immune responses to the payload antigen; however, the use of Ad5-based vaccines is limited for malaria due to the prevalence of pre-existing neutralizing antibodies to Ad5 in sub-Saharan Africa. To avoid this issue, we are developing our vaccine based on the adenoviral serotype Ad28 as a platform for adenoviral-vectored vaccines. We have found that Ad28 generates stronger immune responses than other alternative serotypes to Ad5, such as Ad35. Recent studies in non-human primates and in humans suggest that a multivalent vaccine may be more effective at protection than vectored, single-antigen vaccines. Along these lines, we have developed a stable, multiply deleted, Ad28 vector that contains multiple *Pf* antigens. We and others have noted that vaccines based on alternative serotypes have generally under-performed Ad5 vectors with regard to induction of antigen-specific immune responses. To address this issue, we are investigating several fiber modifications to the Ad28 fiber that have been shown to increase alternative serotype immunogenicity. In conclusion, our data shows that a multiply deleted Ad28-based vector generates strong immune responses in mice and has potential as a malaria vaccine

166

AN ALL-SYNTHETIC NANOSPHERE VACCINE TARGETING *PLASMODIUM FALCIPARUM* ENOLASE INDUCES POTENT AND LONG LASTING ANTIBODY TITERS IN MICE

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Enolase catalyzes at the 9th step of the 11 enzymes in the glycolytic pathway. Our field serological studies have suggested that antigens toward *Plasmodium falciparum* enolase were strongly presented by the sera taken from endemic inhabitants who have present and/or recent past infection. To use our findings for vaccine development, we have designed an all-synthetic vaccination material to realize the immunity condition in endemic area, in which residents are sequentially infected and thus sustain immunity against parasite infection. In this presentation, we wish to report (1) nano-encapsulation of a synthetic antigenic peptide based on the enolase, (2) *in vitro* and *in vivo* degradation, and (3) immunological properties of nanospheres. (1) The synthetic antigen consisting of a part of the enolase sequence (22 amino acid residues) was prepared by Fmoc peptide chemistry. The nanospheres were formulated using an oil/water emulsion technique with bioabsorbable synthetic polymers, poly(lactic acid-co-glycolic acid) and poly(vinyl alcohol). The antigen content was adjusted to 4 and 10 μg/mg of the material depending

on the experimental conditions. (2) *In vitro* and *in vivo* degradation of the nanoparticle were observed by monitoring a fluorescence from labeled antigen molecules. In *in vitro*, the antigen was released from the nanospheres slowly and continuously with nearly zero-order kinetics until 40 days. Then, in *in vivo* condition, the antigens were observed even at 28 days after implanting subcutaneously 1 mg of the nanosphere (4 µg antigen) in each nude mouse. (3) Mice were immunized by subcutaneous injection of 5 mg nanoparticle (50 µg antigen). The antibody response of the mice was over 50-fold increase at the 15 weeks if the IgG titer was compared with non-encapsulated control. The titers were increasing through 60 weeks. These results suggest that this synthetic nanoparticle is a promising candidate as a long-lasting antigenic material toward an effective malarial vaccine.

167

INSECTICIDE RESISTANCE IN THE ANTHROPOPHILIC MOSQUITOES *ANOPHELES ARABIENSIS* AND *CULEX QUINQUEFASCIATUS* IN MACHA, ZAMBIA

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The mosquito *Anopheles arabiensis* is the major vector of *Plasmodium falciparum* in Macha, Zambia. The arboviral and filarial vector *Culex quinquefasciatus* is also present in high numbers throughout the Macha region. A major portion of Zambia's current malaria control program relies on long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) with insecticides. Insecticide resistance in mosquito populations has the potential to lessen and even eliminate the effectiveness of these control methods. CDC bottle bioassays and LLIN survival assays were used to characterize the *An. arabiensis* colony established at Macha, and this data was used as a baseline against which to compare field mosquitoes. F1 offspring of field-collected adult *An. arabiensis* from and *Cx. quinquefasciatus* from eggs collected from oviposition traps were tested for insecticide resistance. High levels of resistance to DDT, pyrethroids, malathion, and deltamethrin-treated net material were detected in *Cx. quinquefasciatus*, and low levels of resistance to DDT and deltamethrin-treated net material were detected in *An. arabiensis*. Molecular assays revealed that the knock-down resistance (*kdr*) allele was frequent in the *Cx. quinquefasciatus* population, but further investigation is required to determine the level of this mutation in malaria vectors. Continued monitoring and assessment is necessary in these populations in order to determine levels of resistance and appropriately modify vector control operations.

168

SPECIFIC IMMUNO-EPIDEMIOLOGICAL BIOMARKERS OF EXPOSURE TO *AEDES ALBOPICTUS* AND *AE. AEGYPTI* BITES

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Aedes mosquitoes are among the main vectors of mosquito borne diseases. Both *Aedes* mosquitoes and the mosquito borne diseases that they transmit are currently expanding geographically. This situation stresses the need for accurate monitoring of these vectors populations. We aim to develop new methods to evaluate Human/Vector contact by immuno-epidemiological tools complementary to entomological methods. Specifically our aim is to evaluate human IgG responses to *Aedes albopictus* (La Réunion) and *Aedes aegypti* (Bolivia) salivary proteins, to give insights on the population exposed to *Aedes* bites. Our results indicate that assessing human IgG anti *Aedes* whole salivary proteins by ELISA can be used to detect individual exposure to vector bites and can therefore help to evaluate the risk of pathogen transmission. We observe no systematic IgG cross reaction between *Ae. albopictus* and *Ae. aegypti* salivary proteins.

Western blot experiments also reveal different patterns of immunogenic salivary proteins between these two vectors: we find not only common immunogenic salivary proteins to *Aedes* genera, but also specific immunogenic proteins to *Ae. albopictus* and *Ae. aegypti*. In addition, these characteristics may be used to discriminate exposure to *Aedes* vectors and furthermore to develop specific biomarkers of exposure to *Ae. albopictus* and *Ae. aegypti* bites. Characterization of specific immunogenic salivary proteins of *Ae. albopictus* and *Ae. aegypti* is under investigation. Such biomarkers, specific to *Ae. aegypti* and *Ae. albopictus* bites, could be used for monitoring emerging *Aedes* borne diseases and to evaluate efficacy of vector control programs.

169

EVALUATION OF LONG-LASTING BIOLOGICAL LARVICIDE AGAINST *ANOPHELES* MOSQUITOES IN KENYA

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Synthetic insecticides are the main chemicals for malaria vector control. Biological insecticides are attractive alternatives for larval mosquito control as they are benign to the environment. However, the currently available bio-larvicide formulations have a short effective duration, and consequently larval control incurs a high operation expense due to requirement for frequent re-treatment of larval habitats. Therefore, formulation of biological larvicides that has long-lasting effects is highly desired. A fourStarTM Single Brood Granules (SBG) of *Bacillus thuringiensis israelenis* (Bti) was evaluated under semi-natural and natural conditions in Kenya. This formulation is designed to be effective against mosquito larvae for up to 6 months. In semi-natural habitats containing soil and rain water, second-instar larvae of *Anopheles gambiae* were introduced, and FourStarTM Bti granules dissolved in rain water with appropriate concentrations were added. The number of pupae produced was recorded daily. We found 100% mortality rate within 48 hrs after fourStarTM Bti was dissolved for two months. The field trial in stable and productive natural habitats is currently ongoing.

170

BIOCHEMICAL MECHANISMS INVOLVED IN DDT AND PYRETHROID RESISTANCE IN TRINIDAD AND TOBAGO STRAINS OF *AEDES AEGYPTI*

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The objectives of this study were to investigate the status of the organochlorine dichlorodiphenyltrichloroethane (DDT) and Pyrethoid (PY) resistance in Trinidad and Tobago strains of *Aedes aegypti* and the underlying biochemical mechanisms. Nine strains of *Ae. aegypti* larvae from Trinidad and Tobago were assayed to DDT and PYs (deltamethrin and permethrin) using the Centers for Disease Control and Prevention (CDC) time-mortality based bioassay method. A diagnostic dosage (DD) was established for each insecticide using the CAREC reference susceptible strain and a Resistance Threshold (RT) - time in which 98-100% mortality was observed in the CAREC strain - was calculated for each insecticide.

Mosquitoes which survived the DD and RT were considered as resistant and the resistance status of each field strain was categorized based on the WHO criteria with mortality <80% indicative of resistance. Biochemical assays were conducted to determine the activities of α and β esterases, mixed function oxidases (MFO) and glutathione-S-transferases (GST) enzymes which are involved in resistance of mosquitoes to DDT and PYs. Enzymatic activity levels in each strain were compared with those obtained for the CAREC susceptible strain and significant differences were determined by Kruskal-Wallis and Tukey's non-parametric tests ($p<0.05$). The established DDs were 1µg/100ml, 20µg/100ml and 100µg/100ml for deltamethrin, permethrin and DDT, respectively; and the RTs for deltamethrin, permethrin and DDT were 30, 75 and 120 mins, respectively. All field strains were resistant to DDT (<80% mortality), two strains were incipiently resistant to deltamethrin and three to permethrin (80-98% mortality). Biochemical assays revealed elevated levels of α-esterase and MFO enzymes in all strains. All, except three strains, showed increased levels of β-esterases and all strains, except Curepe, demonstrated elevated GST levels. Metabolic detoxification of enzymes is correlated with the manifestation of DDT and PY resistance in Trinidad and Tobago strains of *Ae. aegypti*. The presence of this resistance also suggests that knock down (kdr)-type resistance may be involved, hence the need for further investigations. This information can contribute to the development of an insecticide resistance surveillance program and improvement of resistance management strategies in Trinidad and Tobago.

171

COMMUNITY USE OF LONG-LASTING INSECTICIDAL NET IN COMBINATION WITH CARBAMATE TREATED PLASTIC SHEETING FOR INSECTICIDE RESISTANCE MANAGEMENT IN MALARIA VECTORS

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Due to the spread of insecticide resistance in African malaria vectors, there is an urgent need to develop alternative tools and strategies for the control and management of resistant mosquito populations. In this context, a new Insecticide Resistance Management (IRM) strategy based on the community use of Long Lasting insecticidal Net (LLIN) and Carbamate-Treated Plastic Sheeting (CTPS) was evaluated in southern Benin. A randomized controlled trial (phase III) was carried out in 21 villages in the district of Tori-Bossito. The impact of a full coverage of LLIN, alone or in combination with CTPS, was investigated in terms of malaria transmission and insecticide resistance management in comparison with a control group (i.e. selective coverage of LLIN to children < 5 following the National Malaria Control Program policy). 55,405 mosquitoes of which 1,713 *Anopheles gambiae* and 1,091 *Anopheles* funestus were collected from July 2008 to December 2009. *Anopheles* funestus density was significantly reduced (about 80%) with LLIN and LLIN+CTPS groups compared to the NMCP group ($P<0.001$). No significant reduction of *Anopheles gambiae* density was however observed with a full coverage of LLIN compared to the control ($P=0.061$), whereas combination of LLIN+CTPS significantly reduced the population size of *An. gambiae* (49% reduction, $P<0.001$). The Entomological Inoculation Rate was reduced by 40% ($P=0.010$) and 70% (P0.05). After 18 months intervention, this frequency increased in all treated arms but the frequency evolved faster with a full coverage of LLIN compared to the combination of LLIN+CTPS ($P=0.005$). Regarding carbamate resistance, the frequency of the ace 1R allele was low in the study site (<10%) but did not increase regardless the treatments ($P>0.05$). This study confirmed previous findings in experimental huts showing that a combination of LLIN and CTPS in a same dwelling is promising for the control and management of pyrethroid-resistant malaria vectors in Africa.

172

EFFICACY OF A MOSAIC LONG-LASTING INSECTICIDE NET (PERMANET3.0) AGAINST WILD POPULATIONS OF RESISTANT *CULEX QUINQUEFASCIATUS* IN EXPERIMENTAL HUTS IN TOGO (WEST AFRICA)

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The efficacy of new Long Lasting Insecticide Net; PermaNet3.0, against *Culex quinquefasciatus* was evaluated in six experimental huts (February-March, 2008) in Lomé (Togo). Endpoints of evaluation were deterrence, exophily, blood feeding inhibition and mortality. Also, wash resistance of the net and its efficacy on vectors was compared with commercially marketed PermaNet2.0 net. In parallel, field susceptibility and resistant status of *Cx. quinquefasciatus* and *Anopheles gambiae* local populations were assessed by testing to Permethrin (1%), DDT (4%), Bendiocarb (0.1%), Deltamethrin (0.5%, 0.05%), Carbosulfan (0.4%) and Chlorpyrifos Methyl (0.4%) using WHO test tubes and protocol. Subsequent evaluation of Kdr status was done in *An. gambiae* s.s. 1,223 *Cx. quinquefasciatus* females were collected in six week evaluation period (one Latin square rotation). The unwashed PermaNet3.0 deterred 16.84% of total *Culex* mosquitoes caught. After 20 washes, the net deterred 5.79% mosquitoes compared to 6.84% by unwashed PermaNet2.0 net. Also, the net induced mosquitoes to exit huts by 50.48% and inhibited blood feeding 70.97% in unwashed state. After 20 washes, the net induced 42.91% mosquitoes to exit and inhibited 67.06% of mosquitoes from blood feeding. The new PermaNet3.0 gave 76% personal protection at zero wash and 69% protection after 20 washes. More so, the net retained almost equal its insecticidal effect at zero wash (7.1%) and after 20 washes (6.5%). In susceptibility test, *An. gambiae* populations showed resistance to DDT, Permethrin and Carbosulfan (12%, 61% and 77% respectively) but susceptible to CM (100% mortality) and Deltamethrin (100% mortality). *Culex quinquefasciatus* species however were resistant to all insecticides tested. M molecular form of *An. gambiae* s.s was predominant (97%) with no S form detected. One hybrid form was detected (3%). The kdr resistant genotype frequency F(R) was 0.84 with 70% homozygotes kdrRR. The evaluation depicts the success of vector control innovations using pyrethriods and non-pyrethriods in combination on nets.

173

MULTIPLEX ASSAY DEVELOPMENT FOR SPECIES IDENTIFICATION AND MONITORING OF KNOCK DOWN RESISTANCE IN *ANOPHELES* MOSQUITO VECTOR POPULATIONS OF PAPUA NEW GUINEA

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Extensive distribution of indoor residual spraying of insecticides and long lasting insecticide treated bednets for prevention of malaria have created selective pressures resulting in the development of insecticide resistant mosquitoes in malaria-endemic regions of the world. A point mutation in the voltage-gated sodium channel gene (*VGSC), kdr,* is the most common variation associated with resistance to DDT and pyrethroid insecticides used in vector control. In the Papua New Guinean *Anopheles punctulatus* (Ap) species complex (>10 species), species-specific insecticide resistance has not been characterized. As morphological species identification has proved challenging within the Ap complex, we undertook DNA sequence-based strategies to evaluate species-specific differences and *kdr* associated polymorphisms. We observed consistent differentiation among Ap, *A. koliensis*, *A. farauti* 1 &4, revealing species-specific ITS2 and VGSC polymorphisms from DNA sequences of 90 mosquitoes in 7 provinces

of Papua New Guinea. To determine if VGSC sequence polymorphisms distinguish Ap sibling species consistent with ITS2 variation, VGSC and ITS2 sequence specific probes were designed and 237 mosquitoes were evaluated. Results showed that all samples were homozygous wild type at the *kdr* mutation site. Results comparing species-specific polymorphisms were 100% (237/237) concordant between the traditional ITS2 marker and the VGSC sequence variants. Together, VGSC and rDNA molecular methods consistently showed that morphological factors are less reliable in identifying species than DNA based analyses due to the cryptic nature of the *Ap* complex. In addition to monitoring for common insecticide resistant mutations like *kdr*, effective vector control programs must have reliable methods of species identification. Our results suggest that the VGSC gene-based assay allows for the simultaneous evaluation of the *kdr* associated genotype and molecular species identification following a single PCR.

174

SUSCEPTIBILITY STATUS OF *AEDES AEGYPTI* TO INSECTICIDES IN LA GUAJIRA (COLOMBIA)

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Dengue fever keeps an endemic behavior in La Guajira, a location in the northern Caribbean coast of Colombia where insecticides have played an important role in actions towards the control of this disease during the last four decades. However, the real susceptibility of the vector mosquito *Aedes aegypti* to insecticides in this location is still unknown. The aim of this work was to evaluate the susceptibility status to insecticides organophosphorus, organochlorine and pyrethroid of three populations of *A. aegypti* in La Guajira-Colombia during the year of 2009. Biological assays were carried out with adults (F2) and third-instare larva of *A. aegypti* collected in different urban districts of La Guajira (Fonseca, Maicao and Riohacha). For adults, the method of the CDC-Atlanta (Centers for Disease Control and Prevention in Atlanta, GA) was applied using diagnostic doses for malathion (100 µg/ml), fenitrothion (75 µg/ml), DDT (150 µg/ml) and lambdacyhalotrine (6,25 µg/ml). For larva, the World Health Organization method was applied, with a diagnostic dose of Temephos (0,012ppm). Each insecticide was tested three times, with four replicates each time, and a control with no insecticide was also included. 100% susceptibility (100% mortality) was observed with malathion and fenitrothion in the three populations evaluated. Variations in susceptibility/resistance with lambdacyhalotrine (52-100% mortality) and temephos (77-99% mortality) were observed. In contrast, a high resistance was observed to Dichlorodiphenyl-Trichloroethane (DDT) in all populations (2-13% mortality). In conclusion, our results suggest some degree of resistance to insecticides in three populations of *A. aegyptis* in La Guajira-Colombia. This might indicate a growing phenomenon of insecticides resistance in this country area.

175

BASIC EFFICACY OF ORAL INSECTICIDES IN TOXIC SUGAR BAITS TO CONTROL SAND FLIES AND MOSQUITOES

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Mosquitoes (Diptera: Culicidae) and sand flies (Diptera: Psychodidae) are vectors of viruses and protozoan parasites. Finding new ways to prevent exposure of people to these hematophagus biting insects and prevent disease transmission is a continuous challenge. Outdoor control measures are an important component of integrated vector management programs. Targeting adult stages is the only option when breeding places are unknown or inaccessible. Conventional control methods, including the use of residual insecticides and thermal fogging, can cause a wide distribution of harmful insecticides and potential exposure to humans and non-target organisms, in addition to limited efficacy in many areas. The use of oral insecticides added to sugar solution is a new, promising alternative method for outdoor control of adult mosquitoes and sand flies. The choice of insecticides is vital for the successful use of the method in the field. Suitable insecticides should be non-repelling even in high concentrations, have good basic oral efficacy on sand flies and mosquitoes, have low toxicity to mammals and other non target animals and remain potent for a reasonable time in harsh outdoor conditions. The great potential for the widespread use of toxic sugar baits necessitates the alternate use of several suitable insecticides to prevent or significantly slow down the development of insecticide resistance. We designed experiments to test the palatability and basic efficacies of spinosad, thiamethoxam, dinotefuran and boric acid in sugar baits against representative mosquito and sand fly vector species. The feeding rates on a series of toxin dilutions and the resulting mortality rates up to 72h post exposure will be presented for *Culex pipiens, Anophles stephensi* and *Aedes aegypti* mosquitoes and *Phlebotomus papatasi* sand flies. The suitability of these insecticides for use in toxic sugar baits and the framework for further testing their persistence is the field will be discussed.

176

DIFFERENTIAL BEHAVIORAL RESPONSES OBSERVED IN *AEDES AEGYPTI* IN RESPONSE TO REDUCED COVERAGE AND DOSE OF STANDARD VECTOR CONTROL CHEMICALS IN THAILAND

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A novel Push-Pull strategy for the reduction of dengue transmission is currently being evaluated in Thailand. One component of this strategy relies on exploiting mosquito behavior in response to vector control chemicals to break human vector contact. In order to achieve the maximum response it is critical to select the appropriate spatial repellent and contact irritant compounds and apply at the correct dose. The current study was aimed at determining the behavioral responses of female *Aedes aegypti* in response to candidate spatial repellent (SR) and contact irritant (CI) compounds. In addition, various treated surface area coverages were evaluated in an effort to reduce indoor mosquito densities while reducing chemical use. Insecticide treated material strips in four different surface area coverage ratios (25, 50, 75 and 100%) were placed on the interior walls of experimental huts in either a vertical or horizontal configuration. The materials used and the configuration of placement were based on data generated from baseline studies conducted on the resting behavior of *Ae. aegypti* in the absence of chemical. Data suggest that there are differential patterns of behavior for *Ae. aegypti* females into (SR) and out of (CI) experimental huts depending upon test chemical, dose applied and treatment coverage. Results from this study will guide the implementation of the Push-Pull control strategy by determining the optimum chemical, dose and coverage to achieve maximum disruption of human vector contact.

177

NEAR-INFRARED SPECTROSCOPY AS A COMPLEMENTARY AGE GRADING AND SPECIES IDENTIFICATION TOOL FOR AFRICAN MALARIA VECTORS

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Near-infrared spectroscopy (NIRS) was recently applied to age-grade and differentiate laboratory reared *Anopheles gambiae sensu strico* and *Anopheles arabiensis* sibling species of *Anopheles gambiae sensu lato*. In this study, we report further on the accuracy of this tool in simultaneously estimating the age class and differentiating the morphologically indistinguishable *An. gambiae s.s.* and *An. arabiensis* from semi-field releases and wild populations. Nine different ages (1, 3, 5, 7, 9, 11, 12, 14, 16 d) of *An. arabiensis* and eight different ages (1, 3, 5, 7, 9, 10, 11, 12 d) of *An. gambiae s.s.* maintained in 250 x 60 x 40 cm cages within a semi-field large-cage system and 105 female wild *An. gambiae s.l.,* were included in this study. NIR classified female *An. arabiensis* and *An. gambiae s.s.* maintained in semi field cages as < 7 d old or ≥ 7 d old with 89% (n=377) and 78 % (n=327) accuracy, respectively and differentiated them with 89% (n=704) accuracy. Wild caught *An. gambiae s.l.* were identified with 90% accuracy (n=105) whereas their predicted age were consistent with the expected mean chronological ages of the physiological age categories determined by dissections. These findings have importance for monitoring control programmes where reduction in the proportion of older mosquitoes that have the ability to transmit malaria is an important outcome.

178

PREDATION EFFICIENCY OF *ANOPHELES GAMBIAE* LARVAE BY AQUATIC PREDATORS IN WESTERN KENYA HIGHLANDS

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The current status of the insecticides resistance in mosquitoes and effect of insecticides on non-targeted insect' species raised the need of alternative control methods for malaria vectors. Predation has been suggested as one of the important regulation mechanisms for malaria vectors in long-lasting aquatic habitats, but the predation efficiency of the potential predators is unknown. In the current study, we examined predation of backswimmer, tadpoles, Gambusia, Belostoma and dragon nymph on *Anopheles gambiae* larvae in semi-natural habitats. Predators were sampled from the habitats, and starved for 12 hours before experiments. Third instar larvae of *An. gambiae* were introduced into two types of microcosms at various larval densities, and the number of surviving mosquito larvae was monitored after 24 hours. We found that habitat type, larval density and predator species had positive impact on the predation rate of *An. gambiae* larvae. All predators have shown to be actively nocturnal. These results suggest that larval predators play a role in regulating larval population of malaria vectors. We are currently investigating the impact of predators in natural habitats.

179

INSECTICIDE-TREATED BED NET (ITN) OWNERSHIP, USAGE AND MALARIA TRANSMISSION IN THE HIGHLANDS OF WESTERN KENYA

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Insecticide-treated bed nets (ITNs) are known to be highly effective in reducing malaria morbidity and mortality. However, compliance of ITN use varies among households and such variations in actual ITN usage may seriously limit the potential impact of the nets and cause spatial heterogeneity on malaria transmission. This study examined the ITN usage and underlying factors for among-household variation in ITN use in two highland sites of western Kenya. Cross-sectional surveys were conducted on ITN ownership (possession) and ITN compliance (actual usage) in occupants of randomly sampled houses in the dry and rainy season of 2009. Despite ITN ownerships reached more than 71%, ITNs compliance was low (<40%). There was also a seasonal variation in ITN compliance rate: compliance rate was significantly higher during rainy than dry season (40% vs. 34%). ITNs were perceived as very important for protection against mosquito bites and malaria by the resident during the rainy season when both malaria prevalence (11.8% vs. 5.1%) and vector densities (1.0 female/house vs 0.4 female/house) were significantly higher than dry season. Other important reasons for higher compliance rate during the wet season include: significantly high numbers of nuisance culicine mosquitoes and low indoor temperatures. Malaria prevalence in rainy season was about 30% lower in ITN users than in non-ITN users, but this was not significantly different during the dry season. In conclusion, in the malaria meso-endemic highland regions of western Kenya, compliance with ITN is relatively higher during rainy season than dry season, but the gap between ITN ownership and usage is high. Reasons for this gap particularly during the rainy season are yet to be known.

180

GENETIC VECTOR CONTROL STRATEGIES TO REDUCE THE BURDEN OF MOSQUITO-BORNE DISEASES

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Vector-borne diseases impose an enormous health and economic burden around the world and new methods to control vector populations are needed. The Sterile Insect Technique (SIT) is an area-wide method of biological pest control whereby large numbers of a pest insect are bred, sterilized (currently by irradiation) and then released. The sterile insects mate with wild insects, but no viable offspring result from those matings. The SIT has been successful against agricultural pests, but is not in large-scale use for suppressing or eliminating populations of mosquito disease vectors. This is due in part to technical difficulties with the current technology. Genetic RIDL® technology (Release of Insects carrying a Dominant Lethal) is a proposed modification that involves releasing insects that are homozygous for a repressible dominant lethal genetic construct rather than being sterilized by irradiation, and could potentially overcome some of those problems. Using the arbovirus dengue as an example, I combine a vector population dynamics model with an epidemiological model to explore the effect of a programme of RIDL releases on disease transmission, and investigate the potential cost-effectiveness by applying estimates of the costs of RIDL-based SIT. Through mathematical modelling I find that this genetic control strategy could eliminate dengue from a human community in a timescale within one year, and at lower cost than the direct and indirect costs of disease that would be averted by doing so.

181

BLOOD-FEEDING AND IMMUNOGENIC *AEDES AEGYPTI* SALIVA PROTEINS

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Mosquito-transmitted pathogens pass through the insect's midgut (MG) and salivary gland (SG). What occurs in these organs in response to a blood meal is poorly understood, but identifying the physiological differences between sugar-fed and blood-fed (BF) mosquitoes could shed light on factors important in pathogens transmission. We compared differential protein expression in the MGs and SGs of female *Aedes aegypti* mosquitoes after a sugar- or blood-based diet. No difference was observed in the MG protein expression levels but certain SG proteins were highly expressed only in BF mosquitoes. In sugar-fed mosquitoes, housekeeping proteins were highly expressed (especially those related to energy metabolism) and actin was up-regulated. The immunofluorescence assay shows that there is no disruption of the SG cytoskeletal after the blood meal. We have generated for the first time the 2-DE profiles of immunogenic *Ae. aegypti* SG BF-related proteins. These new data could contribute to the understanding of the physiological processes that appear during the blood meal.

182

THE CONTRIBUTION OF AESTIVATING MOSQUITOES TO THE SUBSEQUENT WET SEASON POPULATIONS IN THE SAHEL

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Persistence of African anophelines throughout the long dry season (4-8 months) where no surface waters are available remains one of the last mysteries of medical entomology. Recent studies demonstrate that aestivation (summer diapause) is one mechanism that allows the African malaria mosquito, *Anopheles gambiae*, to persist in the Sahel. However, migration from distant localities - where reproduction continues year-round - might also be involved. To assess the unique contribution of aestivating adults to the build-up of populations in the subsequent wet season, we compared two villages subjected to weekly pyrethrum sprays throughout the dry season with two nearby villages. We predict that in the treated villages, mosquito density during the subsequent wet season would be lower and it would peak later if most aestivating mosquitoes are killed by the insecticide. We selected four small, isolated villages in the Sahel region of Mali located over 10 km away from the nearest permanent larval site. Monitoring started in September 2009 in all villages. It consisted of pyrethrum spray collections conducted once a month in 25 houses selected at random in each village. Insecticide treatment in treated villages started after all larval sites dried up (December). Treatment consisted of four pyrethrum sprayings in all houses every month throughout the dry season, until the first rain. After the first rain, only monitoring was performed every ten days in all four villages. The mosquito density and composition before, during, and after the dry-season treatment was compared in each pair of treated and untreated villages based on their geographical proximity. Currently (March 2010), the dry season treatments are ongoing and house density is 0-0.04/house in all four villages. The complete results will be presented and discussed in respect to the role of aestivation to the persistence of mosquitoes in the Sahel and their implication for malaria control.

183

SIMULATIONS OF MOSQUITO HOST-SEEKING BEHAVIOR

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Models of disease spread commonly make the assumption that susceptible and infected individuals are homogeneously distributed within a population or within subpopulations that are interconnected on a large spatial scale. The effect of small-scale spatial heterogeneity on disease transmission remains a relatively unexplored area, and may be particularly important in diseases where transmission occurs between members of different species. I present a computational model to explore the effect of small-scale spatial heterogeneity on the encounter rate between mosquito vectors and bird hosts in the context of West Nile virus transmission. The model includes behavioral rules for the motion of host-seeking vectors, a spreading odor plume generated by resting hosts, and non-uniform wind conditions. The behavior of the vectors and the spatial arrangement of the resting hosts are varied to measure the number and distribution of mosquito-bird encounters. The results may be used to modify the transmission parameter in models of disease spread, such as SIR and its variants, in order to account for the effects of small-scale spatial heterogeneity in host distribution and differences in mosquito behavior across species.

184

CONTRASTING EXPERIMENTAL HABITAT OPTIMA FOR *ANOPHELES* AND *AEDES*

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The mosquitoes Anopheles arabiensis Patton (Diptera: Culicidae), a vector of malaria, and Aedes albopictus Skuse (Diptera: Culicidae), vector of Chikungunya and Dengue, are targeted for population control programs, such as the Sterile Insect Technique (SIT). These two species coexist in the same areas in Reunion Island but are usually found in different breeding sites. Studies were conducted to assess their optimal food concentration for development and survival in order to optimize mass rearing processes for conventional SIT and to better understand their habitat limitations. For each species, 32 first instar larvae were reared in Petri dishes filled with 32 ml of deionised water, and fed daily with 640 microliters of different concentration (1, 1.5, 2, 4 and 8%) of a diet developed in the IAEA laboratory. Diet concentration tolerance was different for the two species: 2% appeared to be a maximum for An. arabiensis whereas Ae. albopictus survived well until 4% and was still able to develop at 8%. When food concentration increased, the development duration was slightly increased for An. arabiensis but reduced for Ae. albopictus. For both species and sexes, wing length increased with food concentration. Considering all the parameters, the best food concentration was 1% for An. arabiensis and 2% for Ae. albopictus. The sensitivity to the organic content and concentration of the aquatic environment was different between these two species as substantiated by our results. Indeed, An. arabiensis is usually known as a "clean-water" species whereas Ae. albopictus is a "polluted-water" mosquito which can develop well in water with a high organic content.

In order to determine whether inter-specific interactions would expand the optima of the Anopheles, both species were then reared in the same container in a 1:1 ratio and given 1, 2 or 4% of food concentration. Our results suggest that the development of Ae. albopictus larvae in water that would otherwise be too organic-rich for An. arabiensis, would make the environment suitable.These results are discussed in the context of diet concentration for mass-rearing and are linked to specific ecological capacities in the field. The diet provided turned out to be suitable for both species, optimizing all the developmental parameters recorded here, and would be adaptable to any mosquito species rearing.

185

WATER USE PRACTICES LIMIT THE EFFECTIVENESS OF A TEMEPHOS-BASED *AEDES AEGYPTI* LARVAL CONTROL PROGRAM IN NORTHERN ARGENTINA

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A five-year larval control program based on citywide application of temephos every 3 or 4 months significantly reduced *Aedes aegypti* larval indices but failed to maintain them below target levels in Clorinda, northern Argentina. Reduced residuality of temephos has been proposed as a putative factor limiting control in large tanks, the most productive container type in Clorinda. The duration of temephos residual effects in household-owned water-holding tanks was estimated in two longitudinal studies including 18 and 60 tanks followed up during 5 and 14 weeks, respectively. Temephos was applied using spoons or inside small zip-lock bags. Water samples from the study tanks (including positive and negative controls) were collected weekly and subjected to larval mortality bioassays. The trials were concurrent with larval control actions in the entire study neighborhood. Water turnover was estimated quantitatively at the end of the follow-up by adding sodium chloride to a sample of the study tanks and measuring its dilution 48 hs later. Temephos residuality was much shorter than the expected 8-12 weeks and very heterogeneous between tanks. Its mean duration was 5 weeks (range 2-9 weeks) when applied inside small zip-lock bags and 3.5 weeks (range 0.3-10 weeks) when applied using spoons. Water use practices were found associated with loss of residuality via multivariate GEE models. Tanks filled with piped water had high turnover rates and short-lasting temephos effects, whereas tanks filled with rain water showed the opposite pattern. Larval infestation reappeared nine weeks after treatment, five weeks after loss of residuality and most likely originated from newly-laid eggs. High water turnover occurred because the intermittent piped water service forced many householders to refill their tanks almost every night. Limited field residuality of temephos coupled with incomplete coverage of breeding sites explain the inability of the control program to further reduce infestation levels with a 3-4 month treatment cycle period.

186

CHARACTERIZATION OF SLC7-TYPE AMINO ACID TRANSPORTERS IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

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Mosquitoes are successful as disease vectors because they require vertebrate blood as a nutrient source for egg development. After a blood meal, yolk protein precursor (YPP)-synthesis is up-regulated in the fat body. Amino acid (AA)-transporters, located in the fat body plasma membrane, facilitate blood meal-derived AA import and generate a signal that is transduced to the yolk protein gene via the TOR/S6K signal transduction pathway. YPP gene expression in *Aedes aegypti* is dependent upon the cationic AAs histidine, arginine, and leucine. Arginine is also the precursor to nitric oxide which is an important molecule for the innate immune system of mosquitoes. We identified 68 putative AA transporters in the genome of A. aegypti, eleven members of the subgroup of SLC7-type AA transporters, and five of the subfamily of cationic AA transporters (CATs). We determined fat body expression levels of the eleven SLC7-transporters and found several of them strongly up-regulated after a blood meal. Using RNAi-mediated knockdown and subsequent analyses of reproductive fitness, aging, and immunity we demonstrate the role of SLC7-type AA transporters in adult female *Aedes aegypti*.

187

DISPERSAL OF *CULEX PIPIENS* IN AN URBAN FOCUS OF WEST NILE VIRUS TRANSMISSION: A MARK-CAPTURE STUDY USING STABLE ISOTOPES

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Mosquito movement and dispersal are key determinants of the distribution of arboviruses. Marking and tracking mosquitoes is a long-used strategy to study mosquito dispersal. However, common techniques for marking mosquitoes have significant limitations, reducing their utility for understanding the ecology of certain arbovirus systems, including West Nile virus (WNV). In this study, we deployed isotopic labeling of *Culex* pipiens larvae in aquatic sites and a mark-capture design. We conducted laboratory experiments to identify enrichment levels of 15N-enriched potassium nitrate and 13C-enriched glucose to distinguish marked individuals from natural levels. Mosquitoes reared in enriched environments reached a mean delta 15N of 199.0 and a mean delta 13C of 93.7, compared to natural levels of 4.5 for delta 15N and of -25.5 for delta 13C. The 15N signal maintained its strength up to 25 days post-emergence but the 13C signal declined over the same time period, indicating higher turn-over of carbon than nitrogen during metabolic processes. Stable isotope additions to larval water did not influence mosquito survival or adult body mass. Field deployment of this mosquito mark-capture method in suburban Chicago yielded promising results for measuring arboviral dispersal in an urban landscape. This technique has broad application to the quantification of movement and dispersal of *Culex* pipiens and other disease vectors.

188

PHYLOGENETIC ANALYSIS OF PAPUA NEW GUINEA MALARIA VECTORS (*ANOPHELES PUNCTULATUS* SPECIES COMPLEX) - ASSESSMENT OF BIODIVERSITY AND IMPLICATIONS FOR VECTOR MANAGEMENT

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Limited studies define relationships among Papua New Guinea (PNG) malaria vector mosquitoes, members of the *Anopheles punctulatus* species complex (*A. punctulatus* [Ap]*, A. koliensis* [Ak]*,* morphologically indistinguishable *A. farauti* [Af] 1-8). Understandably, molecular marker studies are necessary to assess biodiversity of PNG *Anophelines*. To examine species relationships we analyzed the internal transcribed spacer 2 rDNA (ITS2 988bp), cytochrome oxidase I (COI 1277bp) and voltage

gated sodium channel genes (VGSC 1537bp; intronic + coding regions containing mutations associated with knockdown resistance [*kdr*]) of 90 mosquitoes collected from 7 PNG provinces. Phylogenetic analysis was performed using Bayesian and maximum likelihood (ML)-based approaches; including *A. gambiae* (Africa) and *A. longirostris* (PNG) as outgroups. Phylogenetic analysis of single nuclear genes (ITS2 and VGSC) supports the existence of multiple species in the Ap complex, with highly significant internal branch support distinguishing species clusters (ML analysis >92%). Analyses of mitochondrial COI showed different arrangements among species clusters and with significantly lower confidence of species relationships (59-63%). Phylogenetic analysis of the three genes concatenated (3802bp) showed conflicting relationship patterns exist when comparing nuclear and mitochondrial genes. Regardless of analysis technique employed, Ap and Af4 were shown to be most closely and consistently related. Placement of Ak and Af1 varied with gene and analysis method. Some Ap, Af1, and Af4 mosquitoes were shown to stray from their respective clusters, suggesting gene flow between these populations. Ak appeared to be the most conserved cluster, containing only and all Ak samples. Distinct phylogeographic partitioning was observed between Af1 samples from PNG mainland and an island province. Overall, these analyses improve evaluation of species diversity and provide a baseline reference for future comparisons following ongoing distribution of insecticide treated bednets, impacting PNG mosquito populations.

189

CREATION OF A TRANSGENIC MOSQUITO STRAIN FOR USE AS A TOOL IN THE MANIPULATION OF NUTRIENT ACCUMULATION IN THE LARVAL STAGE USING THE HEXAMERIN PROMOTER

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Transgenic mosquitoes have been suggested as an alternative strategy to fight vector-borne diseases like malaria and dengue fever. So far, only two promoters are widely used for transgenic mosquitoes: the vitellogenin promoter (expressed in the adult female fat body after a blood meal) and the early trypsin promoter (found in the adult female gut after a blood meal). In order to characterize a promoter for protein expression in the larval fat body we identified a putative hexamerin promoter. Hexamerins are storage proteins that are highly expressed in the fat body of holometabolous insect larvae. We hypothesize that the hexamerin promoter can be used to express proteins in transgenic mosquitoes in a stage-, tissue-, and sex- specific manner. We identified two hexamerin genes in the published genome sequence of A. aegypti and determined their expression profiles. Next, we identified the transcription start sites via RACE PCR. We performed comparative promoter analysis to identify conserved transcription factor binding sites using the MatInspector® software. A 1.5 kbp DNA fragment containing the putative promoter was cloned in the expression vector pGREEN-Pelican. Next we will test the functionality of the putative promoter by incorporating this construct into the genome of Drosophila and A. aegypti and analysis of EGFP expression during postembryonic development. This transgenic line will be used as a tool for the manipulation of nutrient accumulation during the larval stage in mosquitoes by. Nutrient accumulation is important when considering the development of mosquito control strategies such as sterile insect technique.

190

SEROPREVALENCE OF DENGUE ANTIBODIES AMONG 12 -18 YEAR-OLD STUDENTS IN FOUR SECONDARY SCHOOLS IN TRINIDAD

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The aim of this study is to build a dengue seroprevalence data base which can be used to indicate the presence or absence of the circulating infectious agent, among the secondary school population (ages 12-18). This will contribute to evidence-based information to be used in designing a more effective and efficient dengue prevention and control programme. Approximately one hundred and fifty students, between forms 1-6 (ages 12-18), in each of four geographically pre-determined, but randomly selected secondary schools in Trinidad were selected by stratified random sampling. A finger prick was administered by a trained person, subsequent to which a drop of blood (100 microlitres) was placed on a PanBio ICT Rapid Test Card for Dengue. The ICT Cards were then read and the results were recorded. Each ICT card was then re-read by a second person for agreement on the result. Statistical analyses were conducted using the SPSS Software package (version 16.0). Five hundred and ninety eight students between ages 12-18 were selected by stratified random sampling from four geographically predetermined, but randomly selected schools in Trinidad. Of these, two hundred and ninety (48.5%) were positive by PanBio ICT rapid test. While no level of significance was found in the male to female ratio, and by county, a level of significance for positive seroprevalence was found among cases of persons of Indian origin (chi-square value = 0.036; likelihood ratio = 0.031). It was also observed from a crosstabulation between ethic groups and sex, females were more likely to test positive for dengue antigen (chi-square value = 0.028; likelihood value = 0.025). Additionally, seroprevalence rate increased with age (spearman's r value = 0.667; significant 1-tail value = 0.051). In conclusion, a seroprevalence rate of 48.5% is substantial and has critical implications for public health in the light of four circulating dengue viruses in the country. At this level of prevalence, especially among the young age group, one has to be concerned with the high risk of DHF/DSS. Prevention and control efforts should be targeted from a position of integrated management to include re-tooling of current policies, health education, environmental sanitation, source reduction and vector control. Additionally, the concerns of climate change must be well considered in any dengue management strategy. This opens new doors for further investigation and quantification.

191

A SEMI-FIELD, TUNNEL ASSAY FOR THE EVALUATION OF SPATIAL REPELLENTS IN THAILAND

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We describe a semi-field tunnel assay to be used for the evaluation of candidate spatial repellents against mosquitoes. In the absence of a candidate spatial repellent, *Aedes aegypti* released at the midpoint of a 50-m tunnel show relatively equal preference for human "attractants" positioned inside of screened tents at either end of the tunnel. Ongoing studies are determining if equal preference is exhibited by other mosquitoes species. Additionally, the assay is currently being used to evaluate a metofluthrin-based product for its efficacy against *Ae. aegypti.* We propose that this method is an effective, viable alternative to other approaches that are currently employed to evaluate spatial repellents.

192

VECTOR-HOST INTERACTIONS GOVERNING EPIZOOTIOLOGY OF EASTERN EQUINE ENCEPHALITIS VIRUS IN NORTHEASTERN USA

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Recent emergence of eastern equine encephalitis virus (EEEV) in northeastern US, prompted us to renew research on the eco-epizootiology of the virus. Accordingly, we investigated the vector-host interactions and blood feeding behavior of eight mosquito species representing six genera implicated in the transmission of EEEV in New York, Massachusetts, Connecticut, and New Jersey by using a PCR-based assay and sequencing portions of mitochondrial *cytochrome b* gene. Analysis of engorged mosquitoes revealed that *Culiseta melanura* and *Cs. morsitans* acquired blood meals primarily from avian hosts (87-100%). Wood thrushes, American robins, song sparrows, black-capped chickadees, and a few other Passeriformes birds constituted the most common vertebrate hosts suggesting key roles in supporting EEEV transmission. These principally ornithophagic mosquitoes also acquired blood meals from mammals (0.6-4.2%) including humans, and from both birds and mammals (0.3-11.5%) in mixed-blood meals. The frequency of mammalian feedings suggests that *Cs. melanura* and to a lesser extent *Cs. morsitans* may play a role in the transmission of EEEV to equines and humans, in addition to maintaining enzootic transmission among avian hosts. *Anopheles punctipennis*, *An. quadrimaculatus*, *Aedes vexans*, and *Ochlerotatus canadensis* were identified as predominately mammalophagic mosquitoes (92-100%) with no or little inclination for feeding on avian hosts (0-2.5%), or mixed-blood meals (0-6%). *Culex salinarius* and *Coquillettidia perturbans* exhibited a relatively opportunistic blood feeding behavior on avian (36% and 11.8%, respectively), mammalian (53% and 86.7%), and mixed avian-mammalian hosts (11%, and 1.5%). These mammalophagic / opportunistic mosquitoes, may participate as bridge vectors in epidemic / epizootic transmission of EEEV from viremic birds to mammalian hosts. Further details on the resurgence and eco-epizootiology of the EEEV, as well as vectorial capacity of the mosquitoes will be discussed.

193

THE TRANSMISSIVE ROUTE OF *BACILLUS ANTHRACIS* INFECTION

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Many researchers have explained the epizootic spread of anthrax among livestock in pre-vaccinal period by means of activation of infection' carriers, which were considered blood-sucking insects. During a major epizootic of anthrax in Zimbabwe in 1980 amongst wild and domestic animals, accompanied by diseases of over 6000 people, causative vectors of anthrax were flies and gadflies. Epidemiological, epizootological, analytical methods were utilized. Analysis of the spread of anthrax to various natural and geographical areas, epizootic, epidemiological observations suggest that the role of blood-sucking insects in the spread of this disease is possible. Seasonal peak of anthrax animal incidences coincides with the period of maximum activity of arthropods. Anthrax in the past mainly affects horses, cattle, deer, whose predominant the skin carbuncle form. From 1991 to 2009 in various regions of Kazakhstan in 15% cases presumably infection of people with anthrax occurred by the transmissive route. In 1997 in the anthrax foci of Zhambyl region 21 people were infected by anthrax, 107 goals of sheep, 2 horses, 1 pig have fallen. The seven people ill anthrax, were children from 5 to 14 years who had not participating in the slaughter of animals were staying in the anthrax focus. Localization of anthrax carbuncles was not typical for the contact infection route. The carbuncles on the front surface of the tibia, on the flexor surface of the right elbow, on the outer surface of the knee, ankle, on the dorsum of the foot were revealed. In conclusion, the uncharacteristic localization of anthrax carbuncles, presence of the insect bites, and infection with anthrax of children which didn't contact with contaminated objects, but they stayed in the anthrax focus. This is indirect evidence of possible transmission routes of anthrax infection.

194

EFFECTS OF COMBINED SEWER OVERFLOWS ON WATER QUALITY AND *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) ABUNDANCE IN URBAN ATLANTA

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The factors that favor transmission and amplification of west Nile virus (WNV) infection within urban environments remain poorly understood. We have previously reported that urban streams in the city of Atlanta, GA, receiving sewage pulses from Combined Sewer Overflows (CSOs) are productive habitats for larvae of *Culex quinquefasciatus*, the main vector of WNV in the area. Building upon those findings, we longitudinally investigated the impact of CSOs on water nutrient concentrations and *Cx. quinquefasciatus* productivity and compared these measurements with those taken in a non-CSO affected urban stream. From June to October 2008 we quantified the weekly concentration of ammonia, phosphate, nitrate, dissolved oxygen (DO), PH level and the number of immature *Cx. quinquefasciatus* (sum of I-IV instars larvae and pupae) in a total of 10 pools from two urban streams with similar physical (i.e., water-flow, vegetation cover, waterbed) characteristics. A Prokopack mosquito aspirator was used to quantify the abundance of adult mosquitoes within 5 m of each pool. Two thirds of all identified female mosquitoes at the CSO stream were *Cx. quinquefasciatus*, significantly ($\chi 2=5.425$, P |4.94|, $P<0.05$). In contrast, DO level was significantly higher in the non-CSO stream ($W=3.01$; $P<0.05$). Based on a generalized estimating equation model we determined that larval abundance was positively and significantly associated with ammonia, phosphate, nitrate concentrations and negatively associated with DO concentration. Our study provided further evidence on the factors associated with high Cx *quinquefasciatus* productivity in CSOs. Sanitary sewer management plans in more than 700 US communities relying on CSOs has the potential to reduce the risk of WNV transmission.

195

IMPACT OF VIRUS DOSE, EXTRINSIC INCUBATION TEMPERATURE, AND INCUBATION PERIOD ON VECTOR COMPETENCE OF *CULEX NIGRIPALPUS* (DIPTERA: CULICIDAE) FOR WEST NILE VIRUS AND ST. LOUIS ENCEPHALITIS VIRUS

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Culex nigripalpus, a vector of West Nile and St. Louis encephalitis virus (family *Flaviviridae*, genus *Flavivirus*; WNV, SLEV) in the southeastern US, was characterized for vector competence. For WNV, we assessed the impact of virus dose and incubation period (IP), while for SLEV we assessed the impact of dose and extrinsic incubation temperature (EIT). Vector competence was evaluated with chi-square analyses ($P<0.05$) using rates of infection (% virus-positive bodies out of total tested), dissemination (% virus-positive legs out of those infected), and transmission (% virus-positive saliva out of those infected). Virus titer in bodies, legs, and saliva was also tested. *Culex nigripalpus* were fed blood containing a low dose (LD: 6.3 ± 0.01) or high dose (HD: 7.3 ± 0.1) of WNV (logs plaque-forming units (pfu)/mL ± SE) and held at 28°C for IPs of 6 or 12 d. WNV infection rates were high (100%) and not affected by dose or IP. At 6 d, WNV dissemination rates were highest at the HD, but not different between doses at 12 d. Transmission of WNV was only observed under permissive conditions (HD, 12 d) and was low (11%). *Culex nigripalpus* were fed blood containing a LD (4.0 ± 0.1) or HD (4.6 ± 0.1) of SLEV (logs pfu/mL± SE) and held at 25°C or 28°C for 12 d. SLEV infection rates (≥85%) were not affected by dose or EIT. SLEV dissemination rates were lowest at 25°C for each dose group but rates did not differ between doses, showing a greater impact of EIT than dose. SLEV dissemination rates for the LD (91%) and HD (100%) at 28°C were higher than observed for WNV. Transmission of SLEV occurred at only 28°C. The SLEV doses tested were significantly lower than the WNV doses, yet *Cx. nigripalpus* showed higher SLEV dissemination rates under these conditions. *Culex nigripalpus* vector competence for SLEV and WNV is discussed and compared to previous similar studies of *Cx. pipiens quinquefasciatus* SLEV and WNV vector competence. Studies of environmental and biological factors and their influence on vector competence are essential to understand the role of vectors in virus transmission cycles in nature.

196

HEALTH IMPACT ASSESSMENT (HIA) OF INDIRA SAGAR DAM, MADHYA PRADESH: A CASE STUDY

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Building large dams for the generation of hydropower/ irrigation may transform the flora and fauna of the affected areas and can cause some unforeseen adverse impact on the health of the population. The Health Impact Assessment (HIA) of such projects has been made mandatory by World Health Organization. The study on Health impact assessment of Indira Sagar Dam & Resettlement and Rehabilitation (RR) colonies was initiated in January 2004. The entomological and epidemiological data for all the vector borne diseases i.e. malaria, dengue, JE & Filariasis has been collected in seven districts till March 2010. During these surveys, 32 villages, 18 RR centres, 5 Command area villages and 6 Labour Colonies were surveyed. GIS mapping of all 7 districts was done and is being updated regularly. In October 2008, a special survey focussing on Schistosomiasis was also carried out and snail species found, which has been reported to be specific vectors of cattle. No case for dengue, JE and Filariasis was found in the conducted surveys. A total of 151 samples of drinking water were collected from open wells, tube wells, hand pumps and tanks from all the surveyed areas for detection of coliform and other human pathogenic bacteria using HiWater™ Test Kit (HiMedia). Most of the water samples were positive for harmful bacteria. The information of water testing was promptly given to the concerned PHCs for immediate action. After completing each survey meeting was arranged with Vice-Chairman, Narmada Valley Development Authority (NVDA) and State authorities to intimate the survey highlights and suggest mitigation measures i.e. engineering, epidemiological and entomological to control the vector borne diseases. From October 2005, measures were implemented in the field by state Health Department, National Hydro Development Corporation (NHDC) and NVDA e.g. de-weeding in canals, release of larvivorous fish, source reduction, spray/fogging, use of plastic sheet in the canals, mosquito-proofing of houses. IEC activities and engineering workshop were also carried out. Radical treatment was given to all the Pf cases. Due to implementation of these mitigation measures the density of vectors of malaria, dengue, chikungunya, filariasis and JE has shown a remarkable reduction and also the disease. The project is now extended to cover the entire Narmada basin in Madhya Pradesh.

197

ECO-EPIDEMIOLOGICAL DETERMINANTS ASSOCIATED WITH THE RESURGENCE OF EASTERN EQUINE ENCEPHALITIS VIRUS IN THE NORTHEASTERN UNITED STATES

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Eastern equine encephalitis virus (EEEV) is a highly pathogenic mosquito-borne virus in North America. The fatality rate in humans approaches 35% to 75%, and most surviving patients experience mild to severe long-term neurologic sequelae. EEEV activity is most common in and around freshwater hardwood swamps in the Atlantic and Gulf Coast states and in the Great Lakes region where there are abundant populations of the primary mosquito vector, *Culiseta melanura*. Outbreaks in temperate regions have been sporadic, both temporally and spatially, highly focal, and largely unpredictable. During the last six years, the northeastern US has experienced a resurgence of EEEV activity throughout the region, including locations where it had not been previously detected, resulting in severe disease in humans (26 cases with 9 fatalities) and domestic animals (126 cases). The underlying causes associated with the introduction, amplification, persistence, and range expansion of EEEV in the region are explored. Factors examined include: 1) Temperature and rainfall: high fall water table and excessive rainfall during the spring and summer are strongly correlated, 2) Vector abundance and distribution: viral amplification appears to be driven by high *Cs. melanura* populations, 3) Species specific avian-mosquito interactions and virus titers in mosquitoes: blood meal analyses suggest that key bird species such as wood thrush and American robin serve as amplification hosts and based on local feeding habits and virus titers in field-collected mosquitoes, *Cs. melanura* likely serves as both the enzootic and epizootic/epidemic vector, 4) Genetic variation of regional EEEV isolates: phylogenetic analyses indicate regional differences in EEEV isolates in the northeastern US and provide evidence for local overwintering, evolution and extinction of EEEV strains, with periodic reintroduction from southern sources.

198

FHV-B2 PROTEIN EXPRESSION IN MIDGUTS OF TRANSGENIC *AEDES AEGYPTI* MOSQUITOES AFFECTS ARBOVIRUS REPLICATION

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Flock House virus (FHV) is a pathogen of insects. Early in the viral replication cycle, FHV expresses a 12kDa protein named B2. B2 is required by the virus to suppress the RNA interference (RNAi) immune response pathway of infected hosts. Subsequently, B2 has also been shown to suppress RNAi in other animals and plants. B2 specifically binds to long double stranded and short interfering RNA in a sequence independent manner. We hypothesized that B2, expressed in midguts of transgenic *Aedes aegypti* mosquitoes, suppresses the RNAi pathway, therefore lowers the midgut infection and escape barriers for arboviruses. To test the hypothesis, we generated transgenic *A. aegypti* that expresses B2 in the midgut upon ingestion of a bloodmeal. We microinjected a transposable element-based B2 construct into 1820 embryos of the Higgs' White Eye (HWE) strain of *A. aegypti*. Eight transgenic lines were obtained of which, three (B2-133, B2-230, and B2-284) express B2 in the midguts of bloodfed females. When challenged with the recombinant Sindbis virus (SINV) strain TR339, all three lines showed increased midgut and carcass infection rates and higher virus titers compared to HWE, which suggest that expression of B2 in midguts lowers the midgut infection and escape barriers for SINV. Studies to investigate the effects of B2 expression on dengue and chickungunya virus infections in the transgenic mosquito lines are being conducted.

199

NEW LEISHMANIASIS FOCI IN WESTERN GEORGIA

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As the leishmaniasis is the most serious public health emergences exist in Georgia during the past years it is essential the national authorities to be prepared to react decisively and promptly to control them. According to the 2007-2008 leishmanisis vector surveys in Tbilisi active focuses, number of send flies was decreased. The promoting action for this fact was the enhancement of city cleaning service, initiated by city government. The first news about 4 leishmaniasis cases in Georgia was described in 1913, which presumably was first news about this disease in whole Caucasus region. In 1954, in eastern Georgia were registered 540 cases of visceral leishmaniasis (VL). Most of cases occurred in capital of country -Tbilisi. In 2009, out of 169 registered cases of leishmaiasis in the whole country 89 were located in Tbilisi. In Georgia there are 16 species of leishmaniasis vectors - *Phlebotomine* sand-flies. Due to the significant increase of VL cases in Georgia was initiated a BTEP/ISTC project G 1081 with collaboration of NIH during the 4 years (2005-2009). Mentioned project has been implemented in Tbilisi with the following objectives: Determination of the seroprevalence rate of *Leishmania* infection in humans and dogs (stray and pet); Cultivation, preservation, and identification of isolated parasite strains; Surveillance of Phlebotomine sand fly species within active VL foci, including study of their breeding and feeding behavior and identification of potential or proven vectors. In July 2007 three cases of visceral leishmaniasis were detected in Kutaisi, (all in children) which is the second large city of country by its area and population. Later vector survey was held by NCDC. During this survey several species of *Phlebotomus* genus were collected and identified using the morphology ID keys: *Ph. halepensis, Ph. balcanicus, Ph.sergenti,*. All were adults. Since 1957 none of the leishmaniasis vectors were found in western Georgia. Also no cases of this disease were registered in Kutaisi before and there are no references of existence of its vectors either. In conclusion, in Georgia obviously, climate changes, especially the global warming takes place, which likely can have an influence on various pathogens vectors behaviors, their viability, expansion of the area and prolongation of transmission season.

200

BLOOD-FEEDING PATTERNS IN MOSQUITO COMMUNITIES

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The foraging behavior of blood-sucking arthropods is a key biological event that shapes the transmission cycle of vector-borne parasites. It is also a phenomenon within the realm of community ecology, given that blood-feeding patterns of vectors can occur across a community of vertebrate hosts. Although great advances in knowledge of the genetic basis for blood-feeding choices have been reported for selected vector species, little is known about the role of community composition of vertebrate hosts in determining such patterns. Here, we present an analysis of feeding patterns of vectors across a variety of locations, looking at foraging patterns of communities of mosquitoes, across communities of hosts primarily comprised of mammals and birds. Using null models of species co-occurrence, which do not require ancillary information about host abundance, we found that blood-feeding patterns were aggregated in studies from multiple sites, but random in studies from a single site. This finding can be explained by mosquito species in a community relying primarily on host availability in a given landscape, so that contacts with specific hosts will be influenced more by the presence/absence of hosts than by innate mosquito choices. This host-feeding strategy is a function of blood-feeding plasticity, a key trait of mosquitoes that can explain the emergence of many zoonotic mosquito transmitted diseases. From an epidemiological perspective our findings support the idea that phenomena that enhance synchronization of vectors and hosts can promote the emergence of vector-borne zoonotic diseases, as suggested by observations on the linkages between deforestation and the emergence of several human diseases.

201

MOLECULAR DETECTION AND CHARACTERIZATION OF A *WOLBACHIA* ENDOSYMBIONT IN *AMBLYOMMA AMERICANUM* (IXODIDA: IXODIDAE) IN MARYLAND, USA

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Amblyomma americanum, the lone star tick, is very widespread in the United States ranging from Texas to Iowa in the Midwest and east to the Atlantic coast. It is a vector pathogens causing human granulocytic ehrlichiosis, canine and human granulocytic ehrlichiosis, tularemia, and is associated with Southern tick-associated rash illness. *Wolbachia* is a very common endosymbiont of arthropods and filarial nematodes. We identified a *Wolbachia* infection associated with lone star ticks at six locations in Maryland. We screened 92 adults and 10 pools of nymphs (10 nymphs/pool) using multiple primer sets. *Wolbachia* prevalence was low in screened ticks: one adult (1.8%) (n = 92) and two nymphal pools (2 of 10) were infected. The *Wolbachia* strain was characterized using multilocus sequence typing (MLST) with ftsZ, CoxA, GatB, HcpA, and FbpA genes. A phylogenetic tree was constructed using concatenated MLST gene sequences (2019 bp) and indicated that the *Wolbachia* infection of *A. americanum* belonged to supergroup F.

202

DEVELOPMENT OF A MOLECULAR TAXONOMIC KEY FOR THE IDENTIFICATION OF SCRUB TYPHUS VECTORS, MITES WITHIN THE GENUS *LEPTOTROMBIDIUM*

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Larval trombiculid mites (chiggers) are important vectors of scrub typhus within Thailand and much of Asia. Identification of mites species is extremely difficult and we have proposed to develop a molecular taxonomic-key for the precise identification of trombiculid mites using the Cytochrome oxidase subunit I (COI) gene of mitochondria. Our aim to develop mtDNA barcoding is to identify the pre-defined species of mites collected from field sites, focusing mostly on the reservoir mites for scrub typhus. Phylogenetic analysis (neighbor-joining algorithms, 1000 bootstrap, Phylip program) of *Leptotrombidium* mites from colonies maintained at AFRIMS was performed using full-length sequence of the mitochondrial COI gene. Five species of *Leptotrombidium* mites, *L. chiangraiensis*, *L. imphalum*, *L. fletcheri, L. deliense, L. scutellare,* are maintained in our lab. Full length sequencing of the COI gene was completed for 66 samples from 5 chigger species and compared to *Leptotrombidium* mite COI gene sequences retrieved from GenBank. Results showed that the deduced amino acid sequence of full-length COI revealed the greatest variation and diversity which can be used to discriminate these five chigger species from each other. Results of the phylogenetic tree and concordance with conventional microscopic species identification were discussed. We are working to develop this tool to allow identification of *Leptotrombidium* species from throughout the region to facilitate improved scrub typhus research in endemic regions.

203

DISTRIBUTION AND IDENTIFICATION OF *ANOPHELES BARBIROSTRIS/CAMPESTRIS* AT SA KAEO PROVINCE IN THAILAND

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Elucidating vector distribution based on accurate species identification is important for understanding the nature of the species complex and to achieve vector control. The adult morphologies of both *Anopheles campestris* and *An. barbirostris* are difficult to distinguish due to their resemblances. The pupal skins provide better and more reliable stage characteristics for identification of both species. Pupal skin combined with geographical information systems was used to determine the distribution of *An. campestris* and *An. barbirostris*. Breeding habitats were investigated in malarious areas of Sa Kaeo province, Thailand. *Anopheles* larvae were collected from 89 breeding places in five districts (Aranyaprathet. Watthana Nakhon, Khlong Hat, Khok Sung, and Ta Phraya). Seventy six percent of *An. campestris* and *An. barbirostris* collected by larval sampling were correctly identified using pupal skins and 24% misidentified or ambiguously identified based on adult morphology. *An. campestris* larvae collected in a single habitat ranged between 1-15 larvae with a maximum at Nong Yah Plong village, Pa Rai sub-district, Aranyaprethet district. *An. barbirostris* ranged between 1-4 larvae with a maximum at Nong Mak Fai village, Nong Mak Fai sub-district, Watthana Nakhon district. *An. campestris* larvae were found in high numbers in non-drainage habitats, consisting of swamps, flooded areas, and a ground pool. Furthermore, they were found in a pH range of 6.6-7.5, nitrate nitrogen range 5-10 ppm, phosphorus level ≤ 24 ppm, aluminum level ≥ 80 ppm, calcium level ≥ 3,000 ppm, ferric iron level > 25 ppm, humus level < 3 levels, magnesium level 25-79 ppm, manganese ≤ 24 ppm, sulfate < 1,000 ppm, and potassium level 220 ppm. Soil analysis of *An. campestris* breeding habitats, when compared with a previous study at Tak province, showed that a higher proportion was present in non-drainage and semi-drainage habitats, and a pattern of nitrate nitrogen (5-10 ppm), and aluminum (> 80 ppm). A lower proportion was observed in ferric iron < 7.5 ppm. The potassium level was double at Sa Kaeo (225.7 ± 78.2 ppm.) than of Tak province (105.5 ± 66.8 ppm).

204

THE ECOLOGY OF CUTANEOUS LEISHMANIASIS IN ISRAEL: DEMOGRAPHIC AND SPATIAL ASPECTS OF THE VECTOR - RESERVOIR HOST RELATIONSHIP

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Understanding of the ecology of the wildlife reservoir - disease vector interaction is essential for the control of vector-borne zoonotic diseases. In southern Israel cutaneous leishmaniasis is caused by *Leishmania major*, transmitted by *Phlebotomus papatasi*, and maintained by the sand rat *Psammomys obesus*. In this study, I focused on the demographic and spatial aspects of this interaction. With respect to the demographic aspect, using a 1.5 years mark-release-recapture study, I studied the temporal dynamics and distribution of the disease within the host. Most of the transmission occurred following sand fly activity peak of May. Prevalence increases with age but does not differ between sexes. Survival rate is affected by infection and gender: non-infected females have higher survival rate than non-infected males but vice-versa with respect to infected animals. The probability for an individual host to survive long enough to constitute a potential infection source was estimated as 8.2%. With respect to the spatial aspect, I manipulated the degree of burrow isolation by placing artificial burrows at various distances from active host burrows and monitored the rates of their re-colonization by dispersing sand flies. Artificial burrow colonization rates were highest at 0 and 60 meters but even the farthest burrows at 120 and 240 m were frequently colonized. I also conducted a large-scale survey of sand rat burrow distribution after which I trapped and removed sand rats from selected burrows and after three months monitored burrow re-colonization. I used logistic regression to analyze of the relations between the densities of neighboring active host burrow on infection occurrence per host at various spatial scales. Only at the scale of 500-m radius from host burrow, I found significant positive relations indicating that this is a relevant scale for transmission. A risk calibration model, derived from the equation of the logistic model, suggests that even complete host eradication will not nullify transmission risk thus questioning the benefit of local host eradication strategy. The majority of colonizers were juvenile rodents. Burrow re-colonization is dictated by the phenological state of the Chenopodiaceae plants neighboring the burrow. Results indicate that sand flies, more than sand-rats, are responsible for the spatial dynamics of the disease.

205

ECOLOGY AND SPATIAL DISTRIBUTION OF BREEDING SITES OF *ANOPHELES* LARVAE IN LARACHE PROVINCE, MOROCCO

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Our study was conducted to characterize larval habitats of Anopheline mosquitoes and to estimate the key ecological factors associated with this mosquito's distribution. The study was carried out during June and July 2009 within 25 localities belonging to 10 sectors of Larache province. The aquatic habitats were sampled by standard dipping techniques. The

habitats were characterised based on depth, pH, temperature, oxygen dissolved, conductivity, salinity, distance to the nearest house, algae and emergent plant (presence or absence), turbidity and habitat type. A total of 54 aquatic habitats consisting of swamps, rivers and rice fields were chosen. Fifty-two percent of all habitats samples were positive for *Anopheles* larvae. From all mosquito larvae gathered, 1145 *Anopheles* larvae were collected, from which 381 (28 %) were early instars and 829 (72%) were late instars. Morphological identification of the III and IV instars larvae revealed that 76 % (n=629) were *An. maculipennis sl* and 24 % (n=200) are *An. cinereus.* The only species belonging to *An. maculipennis* complex was *An. labranchiae*. Statistics analysis showed that the density of *An. labranchiae* was associated with turbidity and depth in aquatic habitats. These findings suggest that the distribution of *An. labranchiae* were driven by different environmental factors. Understanding the relationship between habitats, environmental factors and abundance of *Anopheles* larvae is essential for an efficient application of mosquito control methods.

206

EVALUATION OF A METOFLUTHRIN FAN VAPORIZER DEVICE AGAINST PHLEBOTOMINE SAND FLIES (DIPTERA: PSYCHODIDAE) IN A CUTANEOUS LEISHMANISIS FOCUS IN THE JUDEAN DESERT, ISRAEL

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Leishmaniasis is a serious public health problem globally and is also a current operational military threat to US military forces deployed in the Middle East and southwestern Asia. *Leishmania* parasites are transmitted by sand flies (Diptera: Psychodidae). In the Judean Desert in Israel, *L. tropica* is vectored by *Phlebotomus sergenti* sand flies that live in rocky hillsides and travel to residential areas at night in search of a blood meal. There is no prophylactic vaccine available for leishmaniasis, so vector control is crucial to prevent parasite transmission. In Israel, sand fly control involves spraying large quantities of residual insecticide on house walls and adjacent surfaces, but insecticidal efficacy is reduced by high UV radiation, temperatures and blowing dust. In many areas, people leave their windows open at night and do not use personal protection (e.g. topical repellents) to protect against bites from sand flies that enter houses. Thus, there is a greater need for passive control measures which effectively repel biting sand flies. Fan vaporizer devices that emanate spatially active pyrethroids are promising tools that might provide long-lasting, passive protection against arthropod disease vectors around the world. The main goal of this study was to evaluate the effectiveness of the OFF! Clip-On device (31.2% w/v metofluthrin a.i.) for repellency against phlebotomine sand flies near a residential area in a cutaneous leishmaniasis as a result of *L. tropica* focus in the Judean Desert, Israel. Sand flies were collected outdoors using modified CDC light traps and sticky traps (unbaited or baited with CO2). The results of this study will be discussed in the context of the effect of metofluthrin and spatial repellents on phlebotomine sand flies.

207

EXPERIMENTAL INFECTION AND TRANSMISSION OF *LEISHMANIA TROPICA* BY LABORATORY-REARED PHLEBOTOMUS DUBOSCQI

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This is the first report of laboratory transmission of *Leishmania tropica*, a pathogen causing cutaneous and viscerotropic leishmaniasis, by the sand fly *Phlebotomus duboscqi*. This blood-feeding, anthropophilic, sand fly species is broadly distributed throughout southern Africa, and has been incriminated as a vector of *L. major* in Ethiopia, Kenya, Senegal and Sudan. After colonizing this sand fly species in the lab, we employed the hamster infection model to determine its competence to transmit *L. tropica.* Groups of female sand flies (130-160 flies/group) were fed naturally on infected hamsters, or artificially on blood suspension of infected *L. tropica* tissue amastigotes using a chick-skin membrane apparatus. Samples of blood-fed sand flies from each group were dissected and examined by microscopy at 2, 4, 16, and 20 hrs post-feeding, as well as 1-9 and 11 days post-feeding. Promastigote maturation was observed in 67% (50 of 74) of the artificially infected sand flies, with the promastigotes observed in the thoracic and abdominal midgut at 11 days post-infection. Promastigote infection of the abdominal midgut was observed in 3% (4 of 118) of the naturally infected sand flies. Nine days post-infection, 53% (8 of 15) and 41% (12 of 29) of the remaining blood-suspension infected sand flies were re-fed on 2 uninfected hamsters. Thereafter, we monitored the persistence, dissemination, and visceralization of the parasites in these hamsters. The hamsters were sacrificed at 4 months post-exposure to infected sand flies, and blood, spleen, liver, and bone marrow samples from these hamsters were screened by Polymerase Chain Reaction (PCR). Several of these samples (blood, liver, bone morrow and spleen of one hamster, liver and spleen of the second hamster) were found to be PCR positive for the presence of *L. tropica* DNA. However, no skin lesions developed on these hamsters after being bitten by infected sand flies. Collectively, these preliminary results suggest the potential of *P. duboscqi* to serve as a vector of *L. tropica.*

208

EVALUATION OF *IN VITRO* ANTIMICROBIAL ACTIVITY OF WHOLE BODY EXTRACTS OF *LUCILIA SERICATA* MAGGOTS

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Maggot therapy (MT) has been used for centuries and MT has and is being used extensively in the United Kingdom (UK), Germany, and the United States of America, where sterile maggots are commercially available. Therapeutic maggots used most commonly today are those of the greenbottle fly (*Lucilia sericata*) which only attacks necrotic tissue. The medical literatures have shown that the secretions/extracts of maggots are very effective in the treatment of gram-positive bacterial infections. The aim of this study is to assess the performance of *in vitro* minimal inhibitory concentration from whole body extracts of maggots taken from chronic wounds of treated patients against Gram positive and Gram negative bacteria and also yeast strains. Whole body extracts of maggots harvested by injuring *L. sericata* larvae with a mortar had an inhibitory effect on *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis (mecA)*, *Streptococcus pyogenes*, *Acinetobacter haemolyticus*, moderate effect on methicillin resistant *Staphylococcus aureus* (MRSA), *Enterobacter aerogenes* and no effect on *Enterococcus faecium (VanA)*, *Klebsiella pneumoniae* (ESBL), *Enterococcus faecalis, Pseudomonas aeruginosa,* and *Candida albicans*. Whole body extracts of maggots significantly inhibited the growth of Gram positive bacteria better than Gram negative bacteria and this substance would also be advocated as being useful cost-effective and safe alternative for the management of chronic wound infections because of increasing number and prevalence of antibiotic-resistant microorganisms.

209

CONTINUED INTERRUPTION OF LYMPHATIC FILARIASIS TRANSMISSION ONE YEAR AFTER THE CESSATION OF MDA IN A PREVIOUSLY HIGHLY ENDEMIC AREA OF MALI

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Although cessation of mass drug administration in areas endemic for lymphatic filariasis (LF) is currently recommended when prevalence of microfilaremia reaches < 1% and ICT positivity is <0.1% in children < 5 years of age, the impact of persistent vector infection in this setting is unknown. A pilot study of community-based mass drug administration (MDA) with albendazole and ivermectin was instituted in six *Wuchereria bancrofti (*Wb) endemic villages of the southern part of Mali to provide baseline data and guidance prior to the initiation of the National LF Elimination Program. We have previously reported the results of surveillance performed after 6 rounds of MDA, which indicated persistence of Wb at low levels in the insect vector despite an apparent elimination of infection in human population (0/686 adults positive for microfilaremia and 0/120 children positive by ICT). To assess the durability of transmission interruption in this setting, MDA was stopped in 2 villages (1396 inhabitants) that were free of infected mosquitoes and continued in the remaining 4 villages (3489 inhabitants). Human and vector infection was assessed 12 months after MDA in the 6 villages. Microfilaremia was assessed by finger prick blood collected at night in 800 adults, and circulating antigen (CA) status was determined by ICT in 800 adults and 289 children < 5 years of age, none of the individuals tested was found to be positive for microfilaremia or CA. *Anopheles* vectors were collected monthly by human landing catch from August to December in each village. In all, 1499 mosquitoes were dissected from the untreated villages and 2892 from the treated villages. Two infected vectors were found, one from the untreated villages and one from the treated villages. No infective vectors were detected in any of the villages. These data are consistent with continued interruption of transmission one year after stopping MDA in a previously highly endemic area of Mali with a low level of residual vector infection. A longer follow up period is necessary to confirm the absence of recrudescence.

210

EPITROCHLEAR LYMPHADENITIS INFECTION WITH *DIROFILARIA IMMITIS:* MORPHOHISTOLOGIC FINDINGS

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An unusual presentation of a well preserved worm, in a lymph node, from an Indianapolis dog owner, who presented with an enlarged painful epitrochlear lymph node is presented. This node was biopsied and the histological sections of a preserved worm's internal structure is presented and differentiated from other *Dirofilaria spp.* Human infection by filarial worms is unusual in the US. Pulmonary dirofilariasis is the most commonly reported anatomic location in the United States, and is caused by *D. immitis*, also called the dog heartworm. Diagnosis is by histology and serologic diagnosis has not been helpful. Most *D. immitis* agents found in the lungs are necrotic, poorly preserved, and sometimes calcified. (Figure 1a-x) Here we present a histologically preserved *Dirofilaria* in a lymph node. Humans are incidental hosts in the agent's life cycle and terminal hosts. The lymph node shows a necrotizing granuloma with a worm section in the pink necrotic area. A transverse section through a mature adult male shows a pink wall with smooth muscle and regular transverse ridges. Note the even thickness of the cuticles without spikes, the pathognomonic internal cuticular wedged shaped ridges with lateral cords, a muscular layer and reproductive and intestinal tubules. Note the tall coelomyarian muscles and centrally located intestine and reproductive tubes. Worms are 100-350 um in diamater. Projecting into the central worm cavity are internal longitudinal ridges. Unlike *D. immitis,* the cuticle of *D. tenuis* is relatively thick, and shows prominent spike ridges. *D. repens* infect man also and is a more common cause of subcutaneous filariasis, but it has not been reported from the United States. Infection in man presents as lesions of the skin, conjunctivae, arms or legs but rarely in lymph nodes as illustrated here. *D. tenuis* is the species most frequently encountered in the Southeast United States infecting humans and can cause subcutaneous infections. Infections by these filarial worms is uncommon and it is even more uncommon to find their presence in lymph node tissue. A cutaneous mosquito bite adjacent to the lymph node is the purported route of infection rather than hematogenous involvement; this is unlike the more common pulmonary infections where tissue involvement occurs via the blood route.

211

IMPACT OF A COMMUNITY-BASED LYMPHEDEMA MANAGEMENT PROGRAM FOR LYMPHATIC FILARIASIS IN ORISSA STATE, INDIA

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India has an estimated 7 million people living with lymphedema due to lymphatic filariasis (LF). Lymphedema management has been shown to decrease acute episodes of adenolymphangitis (ADLA) and reduce lymphedema progression, but there are little data on the impact of community-based programs for lymphedema patients. A large community-based lymphedema management program began implementation in Orissa State, India in 2007 through an Indian NGO, the Church's Auxiliary for Social Action (CASA), in consultation with CDC. The program relies on health supervisors and village volunteers to teach lymphedema management techniques to affected patients and has scaled up to enroll over 15,000 patients. A random sample of 376 patients was followed over 6 months to evaluate the clinical benefits of the lymphedema management program. Clinical and questionnaire data were collected at baseline and at 1, 2, 3 and 6 months after the start of the program and were analyzed using longitudinal analysis procedures in SAS 9.2. At baseline, 80 (22.9%) patients reported at least one ADLA episode in the last 30 days compared with 38 (11.7%) at 6 months (P<.0001). Adherence to the program increased over time. At baseline, 185 (55.1%) patients reported washing their legs with soap and water while at 6 months 327 (100%) reported doing so (P<.0001). Sixty six (18%) patients reported treating wounds with antiseptic cream at baseline compared to 188 (57%) at 6 months (P<.0001). A logistic model exploring the effects of program adherence on the odds of at least one ADLA episode in the last 30 days demonstrated an odds ratio for time of 0.86 (95% CI: 0.80, 0.93), indicating that the odds of at least one ADLA episode decreased over time while enrolled in the program. A Poisson model also demonstrated that patients with access to antibiotics (OR=0.64, 95% CI: 0.91, 0.86) had a decreased risk of ADLA episodes. These data highlight the beneficial impact of a community-based lymphedema management program to improve ADLA episodes among lymphedema patients in LF endemic areas.

212

FIRST EVIDENCE OF SPATIAL CLUSTERING OF LYMPHATIC FILARIASIS INFECTION IN AN *AEDES POLYNESIENSIS* ENDEMIC AREA

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Successful elimination of lymphatic filariasis (LF) requires accurate identification of residual foci of transmission and stringent surveillance

strategies to combat potential resurgence. This is challenging in areas where the day-biting *Aedes polynesiensis* resides, such as Samoa, since in previous studies no geographical clustering of infection has been demonstrated. Another challenge for this low prevalence phase is the choice of diagnostic assay as testing for circulating filarial antigen (CFA) or microfilariae (Mf) alone may not have adequate sensitivity. This could be solved by using the commercially available Filariasis CELISA to measure antibody. In the current study 5 Samoan villages were chosen based on previous epidemiological assessments to represent a range of infection prevalences. CFA, Mf, and antibody levels in children ≤ 10 years of age had been recorded and results linked to household of residence and/or primary school of attendance. To ascertain the location of exposure, two scenarios based on potential foci of transmission around communities and schools were explored. "Community-based" analyses revealed significant spatial clusters of households with infected individuals and a relationship to antibody positive children when they were included in the spatial analysis. Similarly, "school-based" analyses revealed significant clusters of antibody positive children and these were related to CFA positive individuals when they were included in the spatial analysis. These promising findings are the first published evidence of spatial clustering of LF in a day-biting Aedes polynesiensis endemic area. The study provides a key insight into the management of residual foci and potential future surveillance strategies.

213

DECREASED PREVALENCE OF FILARIAL INFECTION AMONG DIABETIC SUBJECTS ASSOCIATED WITH A DIMINISHED PRO-INFLAMMATORY CYTOKINE RESPONSE

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Epidemiological and animal studies have shown an inverse correlation between the incidence of helminth infections including lymphatic filariasis (LF) and the incidence of atopy and autoimmunity. However, the interrelationship between LF and Type-1 and Type-2 diabetes (T1DM and T2DM, respectively) in humans is not known and hence, two cross sectional studies to assess the baseline prevalence and the correlates of sero-positivity of LF among diabetic subjects were undertaken in Chennai, India. In the first study, there was a significantly lower prevalence (p=0.026) of LF among T1DM subjects (0%; n=200) compared to non-diabetic subjects (2.6%; n=500) providing validation for animal data showing the protective effect of filarial infection on T1DM. More importantly, in the second study, there was a significantly lower prevalence of LF among T2DM subjects (both newly diagnosed [5.7%; n=158] and those under treatment [4.3%; n=161]) compared to pre-diabetic subjects [9.1%; n=154] (p=0.0095) and non-diabetic subjects [10.4%; n=943] (p=0.0463). Among those with filarial infection, there were significantly lower filarial antigen loads among T2DM subjects compared to non-diabetic subjects (Geometric Mean of 354 U/ml in T2DM vs. 1594 U/ml in non-diabetic subjects; p=0.04). Serum levels of the pro-inflammatory cytokines - IL-6 and GM-CSF were significantly lower in T2DM subjects who were LF positive compared to those who were LF negative. There were, however, no significant differences in serum levels of the anti-inflammatory cytokines, IL-10, IL-13 and TGF-beta between the two groups. Thus, there appears to be a striking inverse relationship between the prevalence of LF and diabetes, which is reflected by a diminished serum pro-inflammatory cytokine response in subjects with diabetes and concomitant LF.

214

SEVENTEEN YEARS OF ANNUAL DISTRIBUTION OF IVERMECTIN HAS NOT INTERRUPTED ONCHOCERCIASIS TRANSMISSION IN NORTH REGION, CAMEROON

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A contentious issue in the field of onchocerciasis elimination is whether annual mass administration of ivermectin for 15 to 17 years is sufficient to eliminate transmission. We conducted a study to evaluate whether there is ongoing transmission in a hyperendemic focus in North Region of Cameroon, where annual treatment has been administered for 17 years. Surveys were conducted in 12 communities having baseline 1991 microfilaria (mf) prevalence data and nodule data for adults and children. In 2009 we returned to these communities and examined 775 adults for mf, 1015 adults for nodules. The 1991 baseline mf data for 107 children was compared with followed up in 157 children in 2009. 1991baseline data from ocular examinations for onchocerciasis morbidity were also available for 6 communities, and we evaluated 472 persons in 8 communities in the follow up survey. We also conducted entomological studies and determined annual transmission potential, vector infection (all larval stages) and infective rates (L3 only), and annual biting rates. In total, 12,107 flies were examined. Mf prevalence among adults decreased from 70%% to 4.8% (p<0.0001). The mf prevalence in children reduced from 18.7% to 1.3% (p<0.0001). In ocular studies, mf in the anterior chamber dropped from 42.7% to 5.5% mf (p<0.0001), and punctuate keratitis from 33.5% to 3.6% (p<0.0001). *Simulium* vector flies showed 2.1% infection rate, 1.4% infective rate, 39% parous rate, and annual biting rate per person of 24,945. An annual transmission potential (ATP) of 136 in the follow up survey was observed; compared to an OCP standard requiring ATP<100 to avoid new eye disease. This study showed that 17 years was not sufficient to interrupt transmission or stop ocular morbidity from onchocerciasis. Ivermectin treatment should continue in order to avoid the risk of recrudescence.

215

GEOSTATISTICAL MAPPING OF THE PREVALENCE OF INFECTION DURING THE ONCHOCERCIASIS CONTROL PROGRAMME IN WEST AFRICA: IMPLICATIONS FOR ESTIMATING THE GLOBAL BURDEN OF ONCHOCERCAL DISEASE

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Previous Global Burden of Disease (GBD) estimations calculated that 459,000 and 389,000 disability-adjusted life years (DALYs) were lost to onchocerciasis in 2000 and 2004 respectively. These figures were calculated using simplified disease models, which ignored important morbidities caused by onchocercal skin disease and epilepsy, and probably seriously underestimated the true disease burden. Major factors influencing the numbers of DALYs lost to an infectious disease are the adequate delineation of the population at risk and defining the prevalence of infection. The latter can be determined using limited survey data. However because survey data are usually biased towards areas with high disease prevalence, the true population at risk is

likely far greater than the survey data suggest. Estimates of the population at risk in areas under the African Programme for Onchocerciasis Control (APOC) are informed by epidemiological mapping of onchocerciasis, and through census data collected through community-directed treatment with ivermectin (CDTI). However, such detailed estimates are lacking for those countries that were covered by the Onchocerciasis Control Programme in West Africa (OCP). Therefore, we present work to map the prevalence of infection and populations at risk for the time periods 1975 (pre-vector control), and 1990 & 2005 (the two time-points required for the present GBD), in the OCP countries of West Africa based on spatial statistical approaches. We use a generalised linear spatial model, to describe the relationship between several environmental variables (such as altitude, land cover, distance to nearest river) and community-level prevalences of infection (obtained from OCP survey data). We use this model to predict prevalence over the entire geographical area covered by the OCP at different time-points during the control program. These estimates will be helpful in the calculation of the GBD due to the various onchocerciasis sequelae identified in the refined disease model for West Africa.

216

ONCHOCERCIASIS ELIMINATION IN ABU HAMED FOCUS, NORTHERN SUDAN: A 2007 ENTOMOLOGICAL SURVEY

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This paper presents baseline data and assessment study of the first onchocerciasis elimination program in East Africa. The study was carried out in the Abu Hamed focus of onchocerciasis in Sudan, which represents the northernmost focus of the disease in the world. The focus is located in the Sahara desert around the town of Abu Hamed on River Nile and about 700km northwest of the nearest disease focus in the east of the country. The isolated nature of the Abu Hamed focus made it an attractive target for the activities of disease elimination in the new government elimination policy announced in 2006. The strategy involves a switch from annual to semi-annual (six monthly) community-directed treatment with ivermectin (CDTI) and monitoring of *Onchocerca volvulus* indicators of infection and transmission in both human and vector populations. Baseline entomological data were obtained concurrent with switching to twice per year treatment during the 2007-2008 breeding season. Using human landing captures, 29,969 *Simulium damnosum* blackflies were collected from two sentinel villages (Mograt and Nady) within the focus. O-150 repeat PCR screening of these blackflies in 203 pools was conducted in 2009, and analyzed by PoolScreen. 2 positive pools among were found among the 102 pools tested from Mograt and no positive pools were detected in Nady. Overall results showed an infection rate of 0.84 infected flies per 10,000 flies (95% confidence interval of 0.0497 - 1.88 per 10000 flies) for the Abu Hamed focus. This infection rate indicates transmission of the parasite in Abu Hamed may be close to the level below which the parasite population is unsustainable prior to shifting of treatment to twice per year. Control activities in other foci in Sudan resulted in moderate reduction of transmission, as indicated by blackfly vector screening assays. A repeat entomological survey is planned for 2010. Overall assessment of the elimination activities and challenges are discussed.

217

PROTECTION AGAINST ACCELERATED ATHEROSCLEROSIS IN A MOUSE LUPUS MODEL BY ES-62, AN IMMUNOMODULATORY FILARIAL NEMATODE PRODUCT

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A feature of systemic lupus erythematosus is development of an accelerated atherosclerosis. The gld.apoE-/- mouse represents a good model for this condition, its absence of functional Fas ligand and apolipoprotein E resulting in development of aggravated lupus-like symptoms and accelerated atherosclerosis. Our previous work has shown that the anti-inflammatory statin, simvastatin can reduce onset of both lupus-like symptoms and atherosclerosis in this model animal. We therefore examined the protective effects of another anti-inflammatory molecule, ES-62, a phosphorylcholine (PC)-containing glycoprotein secreted by the filarial nematode Acanthocheilonema viteae. ES-62 was found to have very little effect on lupus-like symptoms as determined by measuring glomerular tuft volume, glomerular cell infiltrates and serum albumin levels but demonstrated a striking 60% reduction in atherosclerotic lesion area. It is known that T15-type antibodies against PC can protect mice from atherosclerosis and hence as ES-62 is a PC-containing molecule, generation of such antibodies was investigated as a mechanism of action for atherosclerosis amelioration. However, ES-62 did not induce T15-type antibodies and thus its protective effects in this model may reflect its known anti-inflammatory properties.

218

IMMUNOGLOBULIN M AND IGG SUBCLASS RESPONSES AGAINST *WOLBACHIA* PEPTIDOGLYCAN-ASSOCIATED LIPOPROTEIN (PAL) IN PATIENTS WITH BANCROFTIAN FILARIASIS

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The presence of large numbers of *Wolbachia* in filarial nematodes raises questions whether *Wolbachia* -derived molecules may contribute to the pathogenesis in lymphatic filariasis. Moreover, the detections of humoral immune responses against the *Wolbachia* antigens may be useful as the immunological marker(s) of morbidity and infection of lymphatic filariasis. The association between anti-peptidoglycan associated lipoprotein (PAL) antibodies and clinical manifestations was studied in 75 individuals from endemic areas (52 individuals with active infection, 4 individuals with clinical manifestations, and 19 individuals were endemic normals). We found that levels of IgG3 antibodies against rPAL were significantly higher in patients with active infection than the endemic normals ($P=0.003$). Furthermore, anti-rPAL IgG3 antibody levels were significantly higher in microfilaremic patients ($P=0.04$). However, the levels of IgM, IgG2 and IgG4 antibodies against rPAL among all groups were not significantly different ($P > 0.05$). The results of this study demonstrate that anti-PAL antibody responses are associated with the presence of filarial infections in humans but not with chronic filarial morbidity. Our results suggest that detections of anti-PAL IgG3 antibodies may be useful for diagnosis of the *W. bancrofti* infection.

219

GENE EXPRESSION IN THE BACTERIAL ENDOSYMBIONT OF THE FILARIAL PARASITE, *BRUGIA MALAYI*

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The parasitic nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* cause a disfiguring disease in humans known as lymphatic filariasis (LF), which affects more than 150 million people worldwide. An intracellular alpha-proteobacterium of the genus *Wolbachia,* which belongs to the family Rickettsiaceae, is found in all life cycle stages of these nematodes. These bacteria are maternally inherited via the egg and are necessary for normal embryogenesis and survival of the parasite. A complete genome DNA sequence for the *Wolbachia* of *B. malayi* was determined in 2005. We used real-time quantitative RT-PCR for comparison of gene expression of *Wolbachia* between two *Brugia malayi* life cycle stages: the L3 vector larval stage and the L4 mammalian larval stage. *Wolbachia* genes significantly upregulated in the L4 stage compared to the L3 stage may be critical for the survival of *B. malayi* in the human host. We tested the number of *Wolbachia* per worm in the L3 and L4 stages by quantitative RT-PCR. We found that there are 100-fold more *Wolbachia* in L4 worms than in L3 worms. Forty-one of the approximately 800 *Wolbachia* genes were selected for study using three biological replicates. Real-time PCR results showed there are expression differences between the biological replicates, but some genes showed consistent differences in expression between L3 and L4 larvae.

220

MOLECULAR CLONING AND CHARACTERIZATION OF A VESICULAR ACETYLCHOLINE TRANSPORTER FROM *ONCHOCERCA VOLVULUS* AND ITS BIOCHEMICAL CHARACTERIZATION IN *HAEMONCHUS CONTORTUS* WORMS

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Onchocerca volvulus is a human subcutaneous parasitic nematode recognized as the single most common cause of irreversible blindness. No drug currently available is completely safe and effective for mass treatment. The identification of suitable drug targets is essential. Regulated release of acetylcholine for neurotransmission requires the loading of acetylcholine into synaptic vesicles by the vesicular acetylcholine transporter. Vesamicol is a compound that blocks acetylcholine accumulation in cholinergic vesicles by acetylcholine into synaptic vesicles by binding to the VAChT protein. The *O. volvulus* putative vesicular acetylcholine transporter gene was cloned and protein characterized. Total protein from *O. volvulus* and H. contortus worms and rat brain were probed with rat anti-VAChT antibodies from the C-terminus rat VAChT by Western blotting. The protein was characterised biochemically by assessing the effect of vesamicol on motility of H. contortus worms. The predicted partial protein is composed of 404 amino acids and contains 11 conserved transmembrane domains. The cloned cDNA contains the 5' end and forms approximately 75% portion of the gene. The putative protein shows extensive homology to the vesicular acetylcholine transporter, *C. elegans* gene (98%)and closely related to other vesicular acetylcholine transmitter transporters and amine transporters with high conservation within the transmembrane regions with charged amino acids indicating functional significance in substrate transportation. Phylogenetic analysis of VAChTs and MATs clusters the *Onchocerca* protein in the same clade with *C. elegans* showing evolutionary relatedness. The phylogeny revealed that nematodes diverged from the ancestry much earlier in evolution compared to other animals. Since unc 17 mutations protect against organophosphorus toxicity and the Torpedo electric lobe provides extremely dense cholinergic innervation to the electric organ, these relationships support a role of VAChT of *O. volvulus* in neurotransmission. Absence of 70kDa protein band in H. contortus or *O. volvulus* proteins revealed marked variations of the amino acid sequences within at C-terminus. The concentrations of vesamicol and incubation periods caused increased inhibitions of worm motility.

221

SYBR GREEN QPCR ASSAYS FOR DETECTING *WUCHERERIA BANCROFTI* AND *DIROFILARIA IMMITIS* DNA IN BLOOD AND MOSQUITOES

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Molecular detection of filarial DNA can be used to map filarial infections and to progress in disease elimination programs. We previously described a highly sensitive real-time PCR method for detecting *Wuchereria bancrofti* (Wb) DNA in blood and mosquitoes. That test employed an expensive TaqMan probe. This study was performed to compare SYBR Green based qPCR assays with TaqMan assays in terms of performance and cost. SYBR Green qPCR assays for Wb and *Dirofilaria immitis* (Di) DNA employed primers specific for LDR (long DNA repeat, 90bp) and MTR (multiple tandem repeat, 84bp; Genbank number M95173) targets, respectively. We compared assays that employed conventional SYBR Green and Power SYBR Green master mixes for detecting filarial DNA isolated from parasites, infected blood, and vectors. The efficiency of qPCR tests with Power SYBR Green to detect both LDR and MTR was close to 100% and better than that observed with conventional SYBR Green qPCR. Ct values with Power SYBR Green assays were 2 cycles lower (4-fold more sensitive) than conventional SYBR Green assays and comparable to TaqMan qPCR assays for these targets. Melting curve analyses showed uniform results for both LDR and MTR with melting temperatures of 73.4C and 72.2C, respectively. Power SYBR Green tests were specific for detecting LDR or MTR with no signals detected with DNA templates from other filarial nematodes, *Plasmodium falciparum*, *Aedes aegypti*, or *Homo sapiens*. Power SYBR Green and TaqMan LDR assay results were identical for 121 pools of gravid mosquitoes collected in endemic areas (40 positive pools) and for 19 blood samples from infected humans (16 positives by qPCR). The two MTR assays produced the same results for *D. immitis* detecting the DNA present in 1/100th of one microfilaria. Both assays also detected *D. immitis* DNA in laboratory infected mosquitoes both immediately after feeding and for 30 days post blood meal. Additional studies are needed to evaluate the sensitivity of the MTR qPCR tests with field collected mosquito and blood samples. The cost of the SYBR Green and TaqMan qPCR assays is approximately the same including the DNA extraction ($3.4/sample). These results suggest that Power SYBR Green qPCR is a promising alternative to probe-based assays for detecting filarial DNA in blood samples and in vectors.

222

GENDER-REGULATED *BRUGIA MALAYI* GENES HAVE *CAENORHABDITIS ELEGANS* HOMOLOGUES WITH GERMLINE (SPERMATOGENESIS AND OOGENENSIS) OR EMBRYOGENESIS-ENRICHED EXPRESSION

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Recent advances in sequencing of parasite genomes have outpaced progress in annotation. We previously studied gender-regulated gene expression in *Brugia malayi* adult worms. In this study, gender-regulated *B. malayi* expression profiles were cross-referenced with expression patterns reported for the free-living nematode *Caenorhabditis elegans*. 119 of 605 *C. elegans* homologues of Brugia transcripts that are male-upregulated (20%) and 151 of 850 homologs of female-upregulated transcripts (18%) are annotated as "germline-enriched" in C. elegans hermaphrodites producing both sperm and oocytes. In contrast, only 11%

of total number of Brugia transcripts on the BmV2array has C. elegans homologues classified as germline-enriched. Most C. elegans germline-enriched homologues of Brugia male-upregulated transcripts (73%) are associated with spermatogenesis, while 75% of the germline-enriched homologues of Brugia female-upregulated transcripts are associated with oogenesis. Bioinformatics analysis suggests that many of the proteins encoded by spermatogenesis and oogenesis associated genes have binding and catalytic activities. RNA-binding activity is dominant in the oogenesis gene set, and ATP-binding is associated with spermatogenesis. Ninety-eight Brugia gender-regulated genes have homologues that are required for early embryogenesis in C. elegans, and 80% of these are female-upregulated. Several of the female upregulated genes in this group encode proteins involved in protein synthesis, cell division and regulation of transcription (ribosomal protein large subunit family member rpl-27 and rpl-22, 40S ribosomal protein s27, cell division control protein 2, and histone family member (his-35). Male-upregulated genes include actin, alpha tubulin and beta tubulin. In situ localization results for 5 genes were consistent with the predicted functions of these genes in B. malayi reproduction. This study illustrates the value of comparative genomics for improving annotation of nematode genomes and transcriptomes.

223

MOLECULAR BASIS OF ENDOSYMBIOSIS BETWEEN *WOLBACHIA* ENDOSYMBIONT AND THEIR FILARIAL NEMATODE, A ROLE FOR A FILARIAL PHOSPHATE PERMEASE THAT IS UP-REGULATED IN RESPONSE TO *WOLBACHIA* DEPLETION

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Anti-symbiotic approach to control filariasis targeting *Wolbachia* endosymbionts in filarial nematodes has met with promising success, but still the molecular basis governing the endosymbiosis between *Wolbachia* and their filarial host remains unclear. Previously, we found up-regulation of a *Litomosoides sigmodontis* phosphate permease gene (Ls-ppe-1) in response to *Wolbachia* depletion at the m-RNA level and hypothesized that Ls-ppe-1 could have an important role in nucleotide metabolism as depletion of *Wolbachia* induces expression of Ls-ppe-1, perhaps to compensate for lack of nucleotides in the absence of their endobacteria. To test this hypothesis, firstly, the regulation of phosphate permease during *Wolbachia* depletion was studied at the protein level in *L. sigmodontis* and *Onchocerca* volvulus, and secondly, the localization of phosphate permease (PPE) and *Wolbachia* in *L. sigmodontis* and *O. volvulus* were investigated in untreated and antibiotic treated filarial worms. Results show the up-regulation of *L. sigmodontis* phosphate permease (Ls-PPE) both at the m-RNA and protein levels and immunohistology results demonstrate that Ls-PPE is localized to areas of the worms that contain *Wolbachia.* We also found the up-regulation of *O. volvulus* phosphate permease (Ov-PPE) at the protein level during *Wolbachia* depletion by doxycycline treatment of onchocerciasis and Ov-PPE is co-localized to compartments of the worms where *Wolbachia* are in abundance. Up-regulation of PPE in response to *Wolbachia* depletion and co-localization of PPE to *Wolbachia* in filarial worms suggests that PPE could play an important in *Wolbachia* -nematode endosymbiosis and their importance is further indicated in *Caenorhabditis elegans* where knockdown of an orthologous phosphate permease results in embryonic lethality, a phenotype seen when filarial nematodes are depleted of *Wolbachia.* The functions of phosphate permease in the endosymbiosis could involve provision or transportation of phosphate to the *Wolbachia* symbionts, which encode all the genes for the de novo biosynthesis of nucleotides. Further ultrastructural analysis using electron microscopy promises to bring more insight into the molecular interaction between phosphate permease and *Wolbachia* and its role in *Wolbachia* -filarial nematode endosymbiosis.

224

SOCIO-ECONOMIC AND BEHAVIORAL FACTORS THAT INFLUENCE COMPLIANCE WITH MASS TREATMENT IN THE NATIONAL PROGRAMME FOR ELIMINATION OF LYMPHATIC FILARIASIS IN KENYA

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In Kenya, mass drug administration (MDA) for Lymphatic Filariasis using was started in 2002. Based on the 2008 MDA, a cross-sectional study to determine factors influencing compliance was conducted and preliminary results of the data which is still being subjected to further analysis are presented in this paper. Two districts were selected for the study and in each district two locations were selected: one with high and the other with low treatment coverage. The study utilized both qualitative (in-depth interviews and focus group discussions) and quantitative tools (interviewer-based questionnaire). For the quantitative data 965 household heads were selected through simple random sampling. The results indicate that some socio-economic factors influenced compliance levels. In areas of low compliance, 30% of the respondents have a main occupation (business, salaried worker and fishing) indicative of higher income level compared to 16% in areas of high compliance. Land ownership, an indicator of high socio-economic status was common in low compared to high compliant areas (95% and 78% respectively). Non- religious believers were more prominent, 62% in low compared to 38% in high compliant areas. In addition, in areas where there was a high perception of risk, there tended to be better compliance although not statistically significant (56% from high and 45% from low). Correct knowledge on cause of LF was more prevalent, 58% in high compared to 42% in low compliant areas. Attitude towards the drug was more positive in high compared to low compliant communities (61.4% and 38%). Problems related to drug's size, number and taste were significantly associated with compliance, more so in low 61.3% compared to 38.7% in high compliant areas. Dislike for the current drug distribution method was significantly associated with compliance; 72% in low and 28% in high compliant areas. Access to information on MDA which seemed to have been better in high compared to low compliant areas (61% and 50% respectively) was another contributing factor.

The study shows that for MDA to be successful, people need adequate information. Information dissemination methods to non-believers should be explored. Alternative methods of drug distribution in higher income areas should be considered. It is important to invest more in sensitization in higher income households to ensure that there is adequate understanding on need for MDA and counter any fears related to drug use.

225

WOLBACHIA-BASED SUPPRESSION OF AN *AEDES POLYNESIENSIS* FIELD POPULATION: A VECTOR CONTROL STRATEGY TO AUGMENT THE LYMPHATIC FILARIASIS ELIMINATION CAMPAIGN

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Lymphatic filariasis (LF), a painful and disfiguring mosquito-borne disease, is the leading cause of disability in the Western Pacific. Mass drug administration (MDA) has effectively reduced LF prevalence. However, historical evidence suggests that the current effort to eliminate LF in the South Pacific is at risk in some regions if it continues to rely solely upon

MDA strategies. This is because the primary vector *Aedes polynesiensis* is a particularly efficient LF vector in areas of low-level microfilaremia. This negative density-dependant transmission complicates the MDA approach. To strengthen the existing MDA program a vector control program is also needed in some regions of the South Pacific. A possible strategy for vector control is the use of Incompatible Insect Technique (IIT) or cytoplasmic incompatibility (CI) which causes embryonic mortality in crosses between individuals of the same species with different *Wolbachia* infection status. Here we will present the results of *Ae. polynesiensis* population monitoring on multiple islands in French Polynesia, including one island that received multiple, inundative releases of cytoplasmically incompatible males. We will provide data relevant to the production, delivery and assessment of released males. To examine for an impact on the targeted population, field collected females were monitored for egg hatch and insemination; broods that did not hatch were interpreted as having mated with an incompatible male. A successful demonstration is that female sterilization through cytoplasmic incompatible male releases will suppress or eliminate the mosquito population.

226

MULTIPLEX PCR-BASED EVALUATION OF *PLASMODIUM* SPP. AND *WUCHERERIA BANCROFTI* INFECTIONS IN PAPUA NEW GUINEA

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The parasites that cause malaria and bancroftian filariasis often co-infect humans in Papua New Guinea (PNG). Although programs to eliminate these parasites have met with some success, simultaneous surveillance of infection by these parasites in humans has been limited by lack of efficient diagnostic tools capable of detecting infection with both high specificity and sensitivity. To address this problem, a multiplex, post-PCR ligation detection reaction-fluorescent microsphere assay (LDR-FMA) has been developed to diagnose *Plasmodium* spp. and *Wuchereria bancrofti* (Wb) infections simultaneously in human blood samples. In this assay, multiplex PCR is used to amplify *Plasmodium* spp. small subunit rRNA and Wb long DNA repeat sequences, and the resulting amplicons are used to perform species-specific LDR-FMA. We used this assay to analyze genomic DNA extracted from blood samples (N=2,674) collected in a region of PNG co-endemic for Wb and the four malaria-causing *Plasmodium* spp. Previous calculations of the specificity and sensitivity of Wb detection were 0.94 and 0.86, respectively, while those of *Plasmodium* spp. detection were 0.79 and 0.81, respectively. Analysis of the data generated from application of this assay to PNG samples showed that the prevalence of infection with at least one of the five parasite species was 0.877. More specifically, the prevalence of *Plasmodium* species was 0.861, while that of Wb infection was 0.135. Although the prevalence of mixed-species infection was significantly higher than that of single-species infection (0.531 versus 0.346, $p < 0.001$), the parasite assemblages constituting the former infection type did not, collectively, occur at a frequency significantly different than that expected given the distribution of the parasites in the study population. Revealing marked complexity of infection by *Anopheles*-transmitted parasites in PNG, the assay introduced herein provides an important tool for efficient measurement of the impact of such public health interventions as distribution of insecticide-treated bed nets.

227

MELANIZATION IMMUNE RESPONSES BY *ANOPHELES PUNCTULATUS* INFLUENCE THE TRANSMISSION OF *WUCHERERIA BANCROFTI* IN PAPUA NEW GUINEA

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Wuchereria bancrofti is vectored primarily by mosquitoes in the *Anopheles punctulatus* complex in Papua New Guinea (PNG). This mosquito complex consists of ~11 cryptic mosquitoes, and also includes the prominent malaria vectors in this area. In PNG, the interaction of *W. bancrofti* with its *Anopheles* vectors has generally been considered one of facilitation, but there is an unexplained reduction in the expected intensity of mosquito infections when mf densities are relatively high. This reduction in parasite intensity has yet to be investigated with vector competence studies. To further evaluate the interaction between *W. bancrofti* and *An. punctulatus*, mosquitoes were collected in the endemic village complex of Drekikire and individually dissected. A total of 418 *An. punctulatus* were captured in the early morning as they rested inside village homes. Seventy-two of the dissected mosquitoes harbored some stage of *W. bancrofti* (17.2% infected) and a total of 242 parasites were recovered (101 mf, 71 L1s, 56 L2s, and 14 L3s). But of these 72 infected mosquitoes, nearly 50% (35/72) elicited a melanization immune response against these parasites. A total of 54 of the parasites were melanized and killed, representing 22% of the entire parasite population recovered. In addition, 14 of the infected mosquitoes killed all of their parasites, giving us an estimate of a refractory rate of 19.4%. This is one of the few instances where melanization has been shown to function as a primary mechanism controlling resistance in a natural vector population. It is possible that the resistance mechanism might be density dependent and even more robust, but controlled laboratory experiments will be required to address these questions. It also is possible that these infection responses by *Anopheles* against filarial worms might influence transmission dynamics for *Plasmodium* in areas where these parasites are co-endemic.

228

IDENTIFICATION OF BENZOXABOROLE PDE4 INHIBITORS AS A THERAPEUTIC AGENTS FOR THE TREATMENT OF LYMPHATIC FILARIASIS

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Insect bite transmitted filarial helminthic diseases, such as onchocerciasis and lymphatic filariasis are major health issues in tropical areas. Onchocerciasis is the world's second leading cause of infectious blindness, with a global prevalence of 17.7 million people. Lymphatic filariasis affects ~300 million people worldwide. The current therapy, Ivermectin, kills microfilariae but not adult worms (macrofilariae) Adult worms can live for up to 8-10 years, thus treatment must be given on a regular basis to break the transmission cycle. This highlights the need to identify new macroflariasidal medicines. Anacor has developed a library of about 5,000 boron-based compounds of diverse structures; several of these novel small molecules are under clinical evaluation to treat psoriasis (Phase II, a PDE4 inhibitor), fungi (ready for Phase III), bacteria (Phase I), as well as malaria, Chagas' disease and African trypanosomiasis (Pre-IND). Using a novel whole-organism screening platform, developed at the Sandler Center for the trematode Schistosoma mansoni, a sample collection of benzoxaborole compounds was screened. Among the hit compounds we noted a very strong enrichment for inhibitors of human phosphodiesterase-4 (PDE4), suggesting that Schistosomal PDE4 might be the target. These hits invariably caused parasite hypermotility, 1-2 hours

onset, and morphological derangements that led to parasite death, after 4 days of exposure.. We have taken these observations and extended them by showing that adult nematodes, *Brugia malayi* and *C. elegans*, also show a hypermotile phenotype after treatment, also with a 1-2 hours onset. *B. malayi* was killed after 4 days of exposure. Furthermore, members of the catechol family of human PDE4 inhibitors were not able to cause this phenotypic change, possibly indicating substantial differences between the human enzyme and nematode enzymes. These kinds of difference should allow targeting of therapy to the parasite. In this poster the structure activity relationship between phenotype and PDE4 activity will be presented.

229

MONITORING TRYPANOCIDAL DRUG EFFICACY IN EXPERIMENTAL RODENTS USING THE LOOP MEDIATED ISOTHERMAL DNA AMPLIFICATION (LAMP)

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Diagnosis of Human African Trypanosomiasis (HAT) relies on conventional parasitological methods to demonstrate trypanosomes in body fluids. These methods are dogged by low sensitivity, thus leave considerable proportions of undetected infections. For the treated patients, in whom relapsing parasitaemia tends to be even lower, they have to be regularly followed up for a period of 18-24 months to confirm cure. The objective of this study was to determine whether the loop-mediated isothermal amplification (LAMP) method can be used as a tool for monitoring treatment success in rodents. Mice were infected with trypanosomes and treated with Melarsoprol or Diminazene Aceturate. Samples were collected from the mice before inoculation, Pre- and post-treatment. Sampling continued at weekly intervals for 4 months; at each occasion microscopy and LAMP were performed to detect circulating trypanosomes or parasite DNA. LAMP remained positive in mice that eventually relapsed after treatment with 14mg/kg Diminazene Aceturate or 10mg/kg Melarsoprol after 4.3±0.5 and 8.2±3.8 weeks respectively. Positive LAMP signals in mice that were successfully treated lasted for a period of 15.2±0.4 weeks post diminazene treatment or 11.4±2.5 weeks for melarsoprol, after which they disappeared. From this study, the LAMP method appears to be a good tool for monitoring trypanocidal efficacy and could confirm cure within 3-4 months. If this can be reproducible in patients, it could cut costs involved in the hitherto lengthy post-treatment follow-up period. Given that LAMP is highly sensitive and has potential to be applicable at treatment centers with basic facilities, it should be further evaluated in treated sleeping sickness patients.

230

TRYPANOSOMA BRUCEI-INDUCED LEUCOCYTE APOPTOSIS AND TRYPANOSUSCEPTIBILITY IN ANIMALS

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The outcome of trypanosomiasis varies with the host, and understanding the mechanism of trypanotolerance is critical to the development of new control strategies. Apoptosis (programmed cell death) has of recent been implicated in the pathogenesis of several infectious diseases. This study was designed to investigate the relationship between *Trypanosoma brucei* - induced apoptosis and trypanosusceptibility. Experiments were conducted in laboratory animals (rats and rabbits) and goats [red Sokoto (RS) and West African dwarf (WAD)] with differing levels of trypanosusceptibility. The effect of diaminazine aceturate (Berenil®) treatment on the level of apoptosis in *T. brucei* infected trypanosusceptible RS goats. Blood and tissues (bone marrow, spleen, thymus, lymph node and liver) were collected for detection and quantitation of apoptosis using, light microscopy (LM), electron microscopy (EM) and DNA gel electrophoresis. Infection was associated with an increase in apoptotic level in all in infected animals. Levels of apoptosis were highest in the spleen. *T. brucei* infected RS and WAD goats showed a significant increase in blood cell apoptosis from 70 days post-infection ($p=0.0092$ and $p=0.0022$ respectively). RS goats showed more severe apoptosis of blood and tissue cells compared to the trypanotolerant WAD. Compared to RS, WAD showed significantly fewer apoptotic cells in the blood ($p=0.0072$). In general, the peak blood cell apoptosis tallied with peak parasitaemia and the lowest leucocyte count. These imply that trypanotolerance is associated with the ability to control apoptotic event during trypanosomiasis. Binucleated lymphocytes were observed in both breeds of goat and correlated with the degree of apoptosis, though the numbers were significantly higher ($p=0.0154$) in RS. Treatment of *T. brucei*-infected RS resulted in a significant decrease ($p<0.05$) in apoptotic cells in blood and tissues. Overall, this study has provided the first evidence of the relationship between blood and tissue leucocyte apoptosis and susceptibility to *T. brucei* infections. Furthermore, this study also provides the first evidence of peripheral blood binucleated lymphocytes during *T. brucei* infections. Future studies have been designed to identify the molecular mechanisms mediating apoptosis of host cells during trypanosomal infections as they may reveal novel therapeutics and vaccine targets for control of the disease.

231

PROPHYLACTIC STEROID THERAPY FOR SAFE AMBULATORY TREATMENT OF SEVERE MUCOSAL LEISHMANIASIS

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Mucosal leishmaniasis (ML) is characterized by scarce parasite load as consequence of a strong TH1 immune response; however, treatment in cases of laryngeal involvement can trigger a local inflammatory response enough severe to cause airway obstruction and even death. For this reason hospitalization could be necessary to minimized risks associated with therapy initiation. Since ML is a neglected disease, patients with mucosal involvement belong to poor, rural and remote areas. Although ambulatory administration of Amphotericin B is available in our center, costs of hospitalization and risk of nosocomial infections are mayor concerns. Patients with confirmed ML (histopathology, culture or PCR) and severe involvement (hoarseness +/- dyspnea + epiglottic and vocal cord infiltration) received 5 days of prednisone 1mg/Kg/d before start treatment with deoxycholate Amphotericin B (AMB). Hydrocortisone 50mg before and after AMB infusion was applied for the first 5 days. Patients were continuously evaluated (vital signs and oxygen saturation every hour) for the first 48 hours of AMB therapy. In absence of complications during this period, treatment continues without closer follow-up. 11 patients were included; all were male and acquired the infection at the central and south jungle of Peru. Mean age was 44 years (SD: 17) and all had previous CL 16 years ago (IQR:10-20). Patients started their symptoms over the nasal area (obstruction + bleeding) 5 years ago (SD: 2,5) and progressively presented hoarseness. 3 received previous treatment with transient improvement. Physical examination revealed extensive compromised included nasal mucosa, palate, uvula and larynx. Even when hoarseness was present in all and dyspnea was present in 2, oxygen saturation was normal in all of them. No changes in vital signs and no signs of respiratory distress were noted during the first 48 hours of treatment. AMB treatment (without steroids) was continued until reach a cumulative dose of 25mg/Kg. In conclusion, individuals with severe ML can be safely treated with AMB as an out-patient if prophylactic steroid therapy is initiated before AMB initiation and if this is continued for at least five days. This regimen can be instituted in endemic regions without "experience and minimal facilities" decreasing costs and probably risks.