third trimester of pregnancy, were administrated under supervision during antenatal care visit in the controlled IPT group. In the non controlled IPT group, drug was given free to women and it was recommended to take it at home. Women were individually randomized to receive non controlled IPTp (n =130) or controlled IPT (n= 125) with SP. Women were followed up during pregnancy and at delivery. A thin and thick smear was done at the ANC every month and if patient presented signs/ symptoms suggestive of malaria at any time. Low birth weight, defined as below 2,500 g, maternal anaemia, placental malaria infection detected by thick blood smear, Rapid diagnostic Test and Biopsy were assessed. Also antenatal parasite prevalence and delivery outcomes in all parity groups were investigated. The prevalence of anaemia (as defined by a haemoglobin concentration of 11.0 g/dL) at delivery were comparable between the controlled and non controlled groups. Effects on of low birth weight (LBW, < 2.5 kg) were non-statistically significant. The prevalence of LBW was 1.5%(IPTp non controlled group) and 1.6% (IPTp controlled group). Unfavourable pregnancy outcomes, including one abortion was observed on IPTp non controlled group. Placental *P. falciparum* infection was observed in 1.6% (Rapid Diagnostic Test) and 3.1% respectively on IPTp controlled groug and IPTp non controlled group. In conclusion, Intermittent preventive treatment of malaria in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) reduces the incidence of low birth-weight, pre-term delivery, intrauterine growth-retardation and maternal anaemia.

527

INVESTIGATION OF GENETIC MUTATIONS IN *PLASMODIUM VIVAX* DIHYDROFOLATE REDUCATASE AND SUSCEPTIBILITY TO CONVENTIONAL AND NEW ANTIFOLATE ANTIMALARIALS USING A *PLASMODIUM FALCIPARUM* EXPRESSION SYSTEM

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With the high prevalence of *Plasmodium vivax* resistance to antifolates throughout Australasia, it is critical to understand the determinants for resistance and for development of new treatments. Like *P. falciparum*, resistance to antifolates such as pyrimethamine and cycolguanil in *P. vivax*, are caused by point mutations within the parasites dihydrofolate reducatase (DHFR)-thymidylate synthase genes. However several unique mutations have been reported in *P. vivax* DHFR and their roles in resistance to classic and novel antifolates are not entirely clear. We have assessed the *in vitro* expression of the *P. vivax* wild- type and various mutant *dhfr* alleles using both episomal and *piggyBac* transposon integrated *P. falciparum* expression systems and compared the effect of these alleles to susceptibility to antifolates. We show that the *P. falciparum* parasites transfected with wild-type *pvdhfr,* in both expression systems, is as susceptible to classic and novel antifolates as the *P. falciparum* with wild-type *pfdhfr,* while *P. falciparum* parasites transfected with episomal mutant *pvdhfr* are resistant to classic antifolates as mutant *P. falciparum* and are notably more resistant to a novel antifolate drug WR99210. Our results show that episomal expression of *pvdhfr* alleles in *P. falciparum*, a closely related biological system, help identify the role and importance of specific mutations against current and new antifolate treatments and provide a system for the quick assessment of the potency of new antifolate drugs against *P. vivax* with different *dfhr* alleles. Whereas, integrated expression systems would help to provide a more stable and reproducible assessment.

528

CHLOROQUINE-RESISTANT *PLASMODIUM FALCIPARUM* HAPLOTYPES FROM THE BRAZILIAN AMAZON

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Most Brazilian malaria cases occur in the Amazon region and *Plasmodium falciparum* accounts for approximately 15% of those with a focal distribution. *P. falciparum* isolates started to show resistance to chloroquine, a mainstay of Brazilian antimalarial policy, in 1960. CQ resistance has been linked to mutations in the *P. falciparum* CQ resistance transporter (*pfcrt*) with the K76T as the critical event while additional mutations (72-76 residue) enhance the resistance. Our objective was to characterize the geographic and temporal extent of CQ-resistant *pfcrt* alleles in the Brazilian Amazon. For this purpose, we examined 177 *P. falciparum* blood isolates from Para, Amapa, and Rondonia states from the 1980s to 2000s. We used direct sequencing to determine the presence of mutations in *pfcrt* and examined four microsatellite loci that cover 11 kilobases (kb) around *pfcrt.* No ancestral-type parasites (CVMNK) were found and all isolates carried the **S**VMN**T** genotype, which has been reported to be a highly resistant CQ genotype in South America. However, there are two SVMNT alleles, one where S is coded for by TCT and the other where it is coded for by AGT. S_{TCT}VMNT allele was more frequently found. Most alleles share a common haplotype which suggests a common founder allele for these genotypes. In addition, this haplotype is similar to that found in other South American countries. Overall this finding suggests strong CQ selective pressure has fixed SVMNT alleles in these Brazilian states.

529

A PROBABLE CASE OF CHLOROQUINE-RESISTANT *PLASMODIUM VIVAX* IN THE PERUVIAN AMAZON

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Chloroquine is nearly universally used as the first line therapy for *Plasmodium vivax* malaria due to its high efficacy and low cost. However, isolated cases of CQ resistance (CQR) have been reported in South America including two cases in Peru. To date very little has been published on the identification of specific molecular markers in the genome of *P. vivax* that confer CQR. We conducted a 2 years study to evaluate 3 regimens of primaquine (PQ) in the Amazon Basin of Peru. We enrolled 540 patients between the ages of 1 and 77 years. All patients had fever or history of fever, and their geometric mean parasite density was 4 271 parasites/µL. All subjects received directly observed therapy with 25 mg/kg of CQ over three days and 0.5 mg/kg PQ daily for 5 or 7 days or 0.25 mg/kg PQ daily for 14 days. Four out of 540 patients had a recurrence of *P. vivax* parasitemia within 35 days of treatment, indicative of CQ failure. The treatment failures were detected on days 28 (twice), 30 and 32, in patients between 4 and 11 years old, with an average geometric mean parasitemia of 382 parasite/µL on D-F. Only one of the four had a total CQ level of 95 ng/mL whole blood at the time of reappearance of parasitemia; the other three had sub-efficacious levels of total CQ in their blood on D-F. *Pvmdr1* gene sequencing and neutral microsatellite markers analysis were performed to distinguish between recrudescence and reinfection. Two out of four had the same genotype at all loci between D-0 and D-F, including the patient with the blood CQ level of 95 ng/mL. No obvious mutation linked to CQR was seen by direct sequencing of the entire ORFs of *Pvmdr1* and *Pvcrt*.

530

"YEAST OPTIMIZED" *PLAMODIUM FALCIPARUM* CRT (PFCRT) AND MDR1 PROTEINS (PFCRT): PURIFICATION, RECONSTITUTION AND PUTATIVE DRUG BINDING

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Nearly two decades ago, a genetic cross of chloroquine-resistant (CQR) and chloroquine-sensitive (CQS) malaria parasites yielded progeny whose chloroquine-resistance phenotype was dependent on a single locus. It is now known that mutations in a single gene within this locus (*pfcrt*) are primarily responsible for the CQR phenotype. However, mutation and / or increased expression of *P. falciparum* multidrug resistance (PfMDR) protein may subtly alter the degree of resistance to some quinolines, and perhaps other compounds. Multiple isoforms of PfCRT and PfMDR1 protein have been successfully overexpressed in *Pichia Pastoris* using a codon-optimized synthetic gene approach, as reported previously. Recombinant protein has been purified using Ni^{2+} chelation chromatography and reconstituted into proteoliposomes (PLs). Recently, a chloroquine photoaffinity analog has been used to map the chloroquine binding site for PfCRT, as reported previously. In this report, we present results from AzBCQ photolabeling experiments that test if PfMDR1 protein binds quinoline-based antimalarial drugs. Furthermore, we photolabel various combinations of PfMDR1 and PfCRT isoforms to test for relative probe affinity differences.

531

ASSOCIATION BETWEEN SNPS IN TRANSPORTER GENES AND *IN VITRO* REDUCED SUSCEPTIBILITY TO ARTEMISININ DERIVATIVES IN PATIENTS ISOLATES OF *PLASMODIUM FALCIPARUM*

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This study was designed to assess the presence of single nucleotide polymorphisms (SNPs) in selected *Plasmodium falciparum* transporter and *pfATPase6* (SERCA) genes as well as pfmdr1 gene copy numbers in fresh patients' isolates, and their association with *in vitro* antimalarial drug susceptibility. Susceptibility of fresh patients isolates of *Plasmodium falciparum* to antimalarial drugs including artemisinin derivatives (ARTs) was determined using the WHO Schizont inhibition assay. SNPs in parasite transporter genes were detected by PCR followed by sequencing of DNA products, and *pfmdr1* gene copy number in *P. falciparum* was assessed by real-time PCR. *In vitro* susceptibility data were collated with sequences analysis of transporter and *pfATPase 6* genes as well as *pfmdr1* gene copy numbers. DNA sequencing data for SNPs analysis in parasite transporter and *ATPase6* genes as well as copy numbers of *pfmdr1* gene was successful in sixty (60) *P. falciparum* isolates with well defined *in vitro* susceptibility profile to antimalarial drugs. We found associations between SNPs in an ABC transporter (PFE0775c) and reduced susceptibility to ARTs. An association was also found between a 3-bp indel in another transporter gene (PF13-0271) and *in vitro* response to ARTs. No patient isolate of *Plasmodium falciparum* showed increase in copy number of *pfmdr1* or polymorphisms in the SERCA gene. The results from this study suggest that SNPs in *P. falciparum* transporter genes other than *pfcrt* and *pfmdr1* may be involved in reduced susceptibility to ARTs *in vitro*. Further studies are needed to validate these findings.

532

LONG TERM PERSISTENCE OF *PLASMODIUM FALCIPARUM* CLONES AND POTENTIAL BIAS IN MEASUREMENT OF DRUG EFFICACY IN LOW TRANSMISSION AREAS

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Examination of parasite genotypes before and after treatment is frequently used to correct estimates of antimalarial drug efficacy. However, when identical clones are common within parasite populations this may result in overestimation of failure rates because patients may be reinfected with identical clones. We investigated the population structure of malaria parasites in Colombia and use this information to estimate the magnitude of this bias. We genotyped 384 SNPs in *Plasmodium falciparum* infections from the Pacific coast of Colombia from 4 states (Chocó, Valle, Cauca and Nariño) using the Illumina BeadXpress platform. We enriched for variable SNPs by selecting sites that were variable in South American genome sequence data, and genotyping was conducted on DNA from finger prick blood samples following whole genome amplification. These data revealed 48 independent parasite clones found in between 2 and 30 patients. The same clones were found in patients sampled 6 years apart and in all four sampling locations demonstrating long term persistence, and broad dissemination of parasites without effective recombination. We measured the probability of genotype identity in parasites sampled at different time intervals. We found that between 5 and 35% of parasites were identical between parasites collected 1-14 days apart, and that 5-10% of parasites were identical in samples collected 15-100 days apart. Hence, a significant proportion of reinfections are likely to be misclassified as drug failures in these populations. Furthermore the extent of this bias is likely to depend on the level of transmission and recombination. We suggest that PCR correction should be used with considerable caution when correcting drug efficacy rates in low transmission areas.

533

CANDIDATE GENE STUDIES IN LOW TRANSMISSION AREAS: DO SNPS (N326D AND S334N) IN THE *PLASMODIUM FALCIPARUM* CHLOROQUINE TRANSPORTER (PFCRT) UNDERLIE RESISTANCE TO AMODIAQUINE/ DESETHYLAMODIAQUINE?

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Low transmission locations have many advantages for conducting association or candidate gene studies with malaria populations because such populations are predominantly monoclonal and linkage disequilibrium is extensive. However such low transmission populations also have disadvantages because they may show extensive geographical and relatedness structure. In this situation, spurious associations may be generated and real associations may be hidden. In initial work, we detected association between N326D and the novel mutations S334N in the pfcrt gene and *in vitro* resistance to amodiaquine and its metabolite, desethylamodiaquine in parasites from the Colombian Pacific coast (Chocó and Nariño states). We genotyped 384 SNPs polymorphisms using the Illumina VeraCode/GoldenGate technology in samples from the Colombian Pacific coast (Chocó, Valle, Cauca and Nariño states) to evaluate the magnitude of population structure and to try to correct for its confounding effects. Three features of the data reveal strong population structure. First, both STRUCTURE and FST based analyses show geographical differentiation in the parasite population sampled. Second, clonally identical parasites were found in different patients. Third, we

observed a large excess of significant p-values (n=128) between randomly distributed SNPs and *in vitro* resistance to chloroquine, amodiaquine/ desethtylamodiaquine, mefloquine and artemisin derivates. To minimize these potential biases we used three approaches: (1) we collapsed multiply represented genotypes into single genotypes, by averaging the IC50 data, (2) we stratified the parasites into subpopulations and (3) we statistically adjusted test scores to take into account population structure. The results of these analyses will be presented.

534

CHARACTERIZING ARTEMISININ INDUCED DORMANCY IN *PLASMODIUM FALCIPARUM* AND ITS POTENTIAL IMPACT ON TREATMENT EFFICACY

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Artemisinin and its derivatives are being successfully used worldwide where multiple drug resistant falciparum malaria is prevalent. Moreover, this group of drugs provides faster clearance of parasitemia than other known antimalarials. In spite of this remarkable activity ~10% of patients fail treatment if artemisinins are given as a monotherapy to non-immune patients. The recrudescent parasites remain susceptible to artemisinin *in vitro*, suggesting a novel mechanism allowing parasites to tolerate drug treatment. Previous studies have indicated that a possible cause for this phenomenon is the persistence of temporarily growth-arrested parasites (dormancy) following drug treatment. To characterize the artemisinin induced dormancy we determined the duration of growth-arrest and the rates of recovery. Asexual *P. falciparum* ring stage parasites were exposed to different concentrations of dihydroartemisinin (DHA) as a model drug. Parasite growth was measured *in vitro* for several *Plasmodium falciparum* lines from different genetic backgrounds. Our observations show that the parasite development is abruptly arrested for a period of up to 20 days post a single dose treatment before returning to normal growth in all strains tested. The majority of dormant parasites resume growth during the first week and the proportion of parasites recovering is dose dependant. Total recovery rates range from 0.001% to 5.5% after single dose treatment with 20 to 500 ng/ml DHA. Repeated DHA treatment leads to a decrease in total recovery rates and parasite lines from different genetic backgrounds show differences in their rates of recovery and duration of dormancy. The effect of potential companion drugs upon artemisinin induced dormancy has also been investigated and will be discussed.These results imply that artemisinin-induced dormancy may be a key factor in treatment failure. Moreover, the risk of the emergence of parasites resistant to artemisinin is increasing and dormancy may play a role as a possible mechanism for the emergence of artemisinin resistance. Therefore, a better understanding of drug-induced dormancy will provide valuable information for the effective use of artemisinin-combination therapies.

535

ASSOCIATION BETWEEN *PLASMODIUM FALCIPARUM* ABC TRANSPORTERS SNPS AND *IN VIVO* PARASITE CLEARANCE AFTER CHLOROQUINE TREATMENT IN MALI

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Transporters of ABC famailly are important by their number and because of their involvement in different pathological manifestations. Many pathogenic organisms use these transporters to export factors of virulence or to resist to antibiotics. SNPs on their genes are known to confer drug resistance in several organisms. The aim of this study was to determine the association between ABC transporter SNPs and *in vivo* parasite clearance after chloroquine treatment. In Malian village of Kolle, we conducted a chloroquine efficacy study in children less than 5 years following WHO protocol. For all children meeting the inclusion criteria clinical and laboratory observations were made. On DNA extracted from filter paper, mutant forms of Pfcrt K76T, Pfmdr1 N86Y, PfG47 and PfG30 were diagnosed using PCR method. To find out any association of SNPs with chloroquine treatment failure, we compared the prevalence of different SNPs at the enrolment day and treatment failure day. We included 196 subjects, among them 27,5% failed to treatment at day 14. Our analyses have showen that the mutant alleles Pfcrt76T and Pfmd1 86Y were associated with chloroquine treatment failure with respectively $p< 0.0001$ and $p< 0.03$. However, mutant alleles of PfG30 and PfG47 genes were not associated, $p>0.5$. In conclusion, in our study, the role of Pfcrt 76T and Pfmdr1 86Y was confirmed, but we did not find any association between mutant alleles of PfG30 and PfG47 genes and *in vivo* parasite clearance.

536

RESISTANCE SELECTION APPROACH TO IDENTIFY AND VALIDATE NOVEL DRUG TARGETS FOR ANTIMALARIAL DRUG DISCOVERY

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Emergence and dissemination of the human malarial parasites resistant to existing drugs have escalated the need for novel and effective antimalarial chemotherapies. The availability of complete genome sequences of different *Plasmodium* species and comparative bioinformatics have divulged several metabolic pathways for antimalarial drug discovery. We adopted whole cell- and target- based approaches for screening of a large number of small molecules and have identified highly potent bioactives that can be powerful probes of parasite-specific biological processes. Drug resistance selection techniques have been used to investigate the mode of action and molecular targets of small molecules discovered in phenotypic whole cell assays. We have selected *P. falciparum* parasite lines stably resistant to several new chemotypes including compounds of both known and unknown mechanism of action. Selection protocols have been optimized to select for target mutations rather than simple amplification of drug pumps or channels. The genetic changes that confer resistance were then identified by applying genomic approaches including sequencing, gene expression profiling and use of a SNP genotyping array to asses copy number variation. These studies have led to the successful identification of both target mutations and gene copy number variations that change the parasite's susceptibility to small molecules. Combining multiple methods for the analysis of lines selected for resistance has greatly improved the frequency of identification the mechanism of resistance and has facilitated the identification of molecular targets of novel chemotypes.

537

PLACENTAL MALARIA AS A PREDICTOR OF LOW BIRTH WEIGHT AMONG HIV-INFECTED AND UNINFECTED WOMEN IN TORORO, UGANDA

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For prevention of placental malaria (PM) in Uganda, pregnant women receive intermittent preventive therapy with sulfadoxine-pyrimethamine (IPT-SP), but HIV-infected women receive daily trimethoprim-sulfamethoxazole (TS). The risk of placental malaria is increased in the setting of HIV, but data on the prevalence of PM and the association of PM with adverse pregnancy outcomes such as low birth weight (LBW) in HIV-infected women on TS are lacking. We performed a cross-sectional study of HIV-infected and uninfected women (1:3 ratio) delivering at Tororo District Hospital, a region of very high malaria endemicity. PM was diagnosed by Giemsa-stained thick smear and *Plasmodium falciparum* PCR of placental blood. We assessed associations with LBW (<2500 grams) and HIV status. Results: We enrolled 517 women. 94% of women were on recommended chemoprophylaxis (TS for HIV-infected and IPT-SP for HIV-uninfected) and only these women were included in further analyses. Overall prevalence of PM was 8% when defined by positive blood smear and 25% when defined by positive PCR. PM by positive blood smear was associated with LBW among HIV-infected (RR 5.12, 95% CI 2.36-11.16) and HIV-uninfected (RR 2.25, 95% CI 1.07-4.75) women. Placentas which were positive by PCR but negative by blood smear were not associated with LBW in either group. There was no increased risk of PM by positive blood smear (OR 0.89, 95% CI 0.29-2.02) among HIV-infected vs. HIV-uninfected women after controlling for gravidity. Prevalence of PM by blood smear of placental blood was low in this area of high malaria transmission. Overall rates of recommended chemoprophylaxis use were high. PM prevalence was similar among HIV-infected and HIV-uninfected women who were taking recommended chemoprophylaxis. PM by blood smear was associated with LBW. PCR was a more sensitive test than placental blood smear for PM, but PCR positivity without a positive smear did not predict LBW, and the clinical significance of placental PCR positivity remains uncertain.

538

MOTHER'S KNOWLEDGE OF MALARIA PREDICTS ITN USE AND FEVER TREATMENT IN CHILDREN UNDER FIVE YEARS - MALARIA INDICATOR SURVEY, ETHIOPIA, 2007

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In 2006, the Ministry of Health in Ethiopia launched a major initiative to scale up interventions, which has included the distribution of over 20 million long-lasting insecticidal nets, universal access to artemisinin combination therapy (ACT), and the training of over 30,000 village-based health extension workers. A cross-sectional national Malaria Indicator Survey was conducted mainly during the malaria transmission season (October-December, 2007). Using probability proportional to size sampling, 319 enumeration areas (EA) stratified by altitude were selected. Households (HH) in each EA were mapped and 25 HH per EA randomly selected. Multivariate logistic regression analyses were performed to assess mother's malaria knowledge as a predictor for insecticide-treated net (ITN) use and fever treatment in children under five years. A principal components analysis generated a composite score of malaria knowledge for each mother which was then dichotomized. Restricting the analysis to HH with at least one ITN, mothers with higher level of composite malaria knowledge were associated with increased ITN use for her child (Odds Ratio [OR]=1.6; 95% confidence interval [CI] 1.1-2.2). Other significant factors in the model predicting ITN use in children included mother having attended school (OR=1.8; 95% CI 1.3-2.6), living in an urban area (OR=1.7; 95% CI 1.0-2.8), living in a HH sprayed with insecticide (OR=1.5; 95% CI 1.0-2.1), increasing number of ITNs in the HH (OR=1.3; 95% CI 1.0-1.6), and decreasing HH size (OR=0.9; 95% CI 0.8-0.9). Mother's knowledge that mosquitoes transmit malaria (OR=1.8; 95% CI 1.2-2.5) was associated with the child having sought treatment for a fever in the last two weeks. Living in an urban area (OR=2.8; 95% CI 1.7-4.7) and mother having attended school (OR=1.7; 95% CI 1.1-2.8) were also significant factors in the model predicting improved treatment seeking. Notably, wealth index was not associated with either ITN use or treatment seeking. On-going delivery of targeted educational information to mothers can improve ITN use and treatment seeking behavior. Furthermore, other social determinants of health such as women's education and development still remain significant and modifiable predictors of health-related behavior. Efforts to control malaria will be influenced by progress towards broader goals of economic development and improving access to education, especially for women.

539

COMPARATIVE ANALYSIS OF SEQUENCES OF THE RECEPTOR BINDING DOMAIN (F2 REGION) OF *PLASMODIUM FALCIPARUM* EBA 175 DERIVED FROM CHILDREN WITH SEVERE, UNCOMPLICATED AND ASYMPTOMATIC MALARIA IN THE KASSENA NANKANA DISTRICT OF GHANA

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The receptor binding domain of EBA 175 has been mapped to region F2 which binds sialic acid residues on glycophorin A with specificity. Antibodies raised against recombinant F2 have been shown to block red cell invasion by *Plasmodium falciparum* in-vitro, providing support for the development of a recombinant malaria vaccine based on this functional domain. The implication of possible polymorphisms within the F2 domain to the clinical outcome of malaria in Ghana is unknown. A nested polymerase chain reaction (PCR) was used to genotype the F2 domain in samples obtained from children with severe, uncomplicated and asymptomatic malaria in the Kassena Nankana District (KND), an area being characterised for future vaccine trials. The F2 PCR products sequenced were translated and aligned with the amino acids of published P falciparum Camp strain to determine the extent of sequence diversity in the F2 domain in Ghanaian isolates. Twenty samples (10 severe, 7 uncomplicated and 3 asymptomatic) were sequenced from subjects. Common polymorphisms were observed at positions 478, 481, 577, 584, 592, 664, and 716 in the severe and asymptomatic controls. Positions 520 - 570 were conserved regardless of the clinical conditions. Phylogenetic analysis using Neighbor-Joining method in MEGA 4 revealed that out of the ten sequences of P falciparum obtained from the severe samples, 8 clustered with the reference strain. All *P. falciparum* parasites obtained from uncomplicated samples clustered with parasites from the asymptomatic samples. The implication of these associations with clinical condition of malaria is discussed.

540

HOLDING THE LINE: LESSONS FROM MAURITIUS FOR PREVENTING REINTRODUCTION OF MALARIA TRANSMISSION

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There is currently a renewed global focus on the elimination of malaria, with more than 30 countries actively pursuing this goal, including six in sub-Saharan Africa. Experience has consistently shown that even if malaria transmission can be interrupted, it can be challenging to maintain, particularly in areas with high receptivity and vulnerability. Little is still understood about effective strategies to prevent reintroduction of local transmission following elimination, especially in areas of efficient transmission such as sub-Saharan Africa. We, therefore, examined the experience of Mauritius, analyzing technical, operational, and financial components of its prevention of reintroduction program. A comprehensive literature review was conducted and complemented with examination of government documents, visits to key implementing institutions, and interviews with policy makers and operational personnel. Subsequent to malaria elimination by mass residual spraying, the island has continued to have a significant *A. gambiae* population and an average of 50 parasitaemic individuals entering the country every year. Despite this, Mauritius has maintained an absence of indigenous cases since 1997 through ongoing vector control, robust surveillance, and prompt case diagnosis and treatment, among others, for a total annual program cost of $2.7 million ($2.15 per capita). An estimated 40% of annual costs are devoted to surveillance and 24% to vector control. The Mauritian experience indicates that strong leadership and management and substantial predictable funding are critical to overcome challenges and consistently prevent the reintroduction of malaria. Further research is needed on the cost-effectiveness of techniques such as active case detection and border screening to inform the planning of post-elimination programs in current eliminating countries.

541

MALARIA INCIDENCE IN INFANTS IN BANCOUMANA, MALI

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Infants in malaria endemic areas are the primary target for a blood-stage malaria vaccine. To properly plan for Phase 2 trials, the incidence of malaria infection in the target population and factors that may affect malaria incidence must be known to appropriately power the trial and determine the sample size needed. This observational study is being performed to determine the incidence of malaria infection and disease, correlate malaria rapid diagnostic test (RDT) and malaria microscopy results, and determine the effect of hemoglobin type on malaria infection in infants in the area of the Bancoumana Vaccine Center. 205 infants aged 6 weeks to 6 months were enrolled, 105 in 2007 and 100 in 2008 and received a baseline evaluation, 6 monthly visits during the rainy season and weekly home visits. At monthly visits, and if ill, infants were examined and determinations of hemoglobin, blood films, and an RDT for malaria were performed. Malaria treatment was given per Mali national guidelines. Results indicate that the incidence of clinical malaria per infant per season was 0.55 in 2007 and 0.21 in 2008. The incidence of anemia (≤ 8.4 g/dl) per infant per season was 0.31 in 2007 and 0.16 in 2008. The use of Insecticide Treated Nets was 30.5% and 74.7% respectively in 2007 and 2008. The concordance between blood smear and RDT results was 97.5%. Effect of hemoglobin type on malaria infection will be in the final results.

542

THE RELATIONSHIP BETWEEN ANTI-MEROZOITE ANTIBODIES AND PROTECTION FROM *PLASMODIUM FALCIPARUM* MALARIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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The epidemiological evidence for the protective effect of naturally acquired anti-merozoite antibodies against *P. falciparum* malaria is conflicting. Some studies report that antigen specific antibodies are associated with protection, whereas others do not, and study quality and design varies substantially. We performed a systematic review, with meta-analyses, to evaluate the evidence that supports a role for antibodies to specific merozoite antigens in protection from *P. falciparum* malaria in naturally exposed populations. PubMed, Web of Science, Scopus and Google Scholar were searched for population-based prospective studies examining the association of anti-merozoite antibodies with incidence risk of *P. falciparum* malaria. Studies had to attain a minimum inclusion and quality criteria to be accepted for review, including a rigorous definition of symptomatic malaria. The appraisal of each study for inclusion and abstraction of data was carried out independently by two review authors. The literature search identified 70 possible studies according to the title of the paper and information in the abstract. On detailed examination, only 32 fulfilled the initial inclusion and quality criteria. We also obtained data from a further 2 studies which were reanalyzed to meet the inclusion and quality criteria. The 34 studies reported data obtained from 14 separate prospective and 6 separate treatment-to-reinfection studies, the majority of which were performed in Africa; only a small number of merozoite antigens have been well studied. The association between antibody responses to recombinant antigens AMA-1, GLURP, EBA-175, MSP-1, MSP-2 and MSP-3 with incidence risk of *P. falciparum* malaria were examined in 6, 7, 2, 22, 8 and 7 publications respectively. Where there was sufficient data, a summary statistic for each antigen and outcome was calculated using meta-analytic methods. This study provides the most comprehensive review, to date, of the strength of evidence of the association of anti-merozoite antibodies with protection against *P. falciparum* malaria.

543

USING DRIED BLOOD SPOTS TO MONITOR CHANGES IN ANTIBODY LEVELS TO *PLASMODIUM FALCIPARUM* IN A REGION OF DECLINING MALARIA TRANSMISSION

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The Zambia National Malaria Strategic Plan 2006-2011 includes provision of treatment with artemisinin-combination therapy, insecticide-treated nets, and indoor residual spraying. Implementation of this plan has resulted in a significant decline in malaria parasite prevalence and disease incidence. Prior experience with intensive malaria control was followed

by a decline in population immunity as malaria transmission decreased, resulting in populations susceptible to resurgence of disease. Development of simple, field-friendly methods to measure parameters of population immunity to *Plasmodium falciparum* is essential to identify populations at risk of resurgence, particularly in regions where successful malaria control strategies have lowered transmission intensity. We optimized and tested an IgG enzyme immunoassay (EIA) against whole asexual stage *P. falciparum* antigens using plasma extracted from whole blood samples stored as dried blood spots. Samples were collected between April and July 2007 (n = 429) in Macha, Southern Province, Zambia as part of a community-based survey of malaria transmission dynamics. Optical density (OD) values were positively correlated with age, consistent with higher antibody levels with increasing exposure. The mean OD value of children under six years of age having had malaria within 3 months (n = 57) was 0.81 compared to a mean OD of 0.62 for those without recent malaria (n = 51). The association between OD values and recent history of malaria was further investigated in persons with more than one sample using generalized estimating equations methods. After adjusting for age, a positive correlation between mean OD and recent malaria infection was suggested (P = 0.064). Estimates of the antibody levels to whole parasite antigens, using dried blood spots collected in community-based surveys, are directly correlated with age and recent malaria infection. This method may be valuable in monitoring changes in population immunity following successful malaria control interventions and in identifying populations at risk of resurgence.

544

MALARIA AND HIV: RELATIVE RATES OF ASYMPTOMATIC FALCIPARUM GAMETOCYTEMIA AND HIV CARRIAGE IN THE BLOOD DONOR POPULATION IN NYANZA PROVINCE, KENYA

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HIV+ patients have more frequent episodes of clinical malaria and higher parasitemias than HIV- patients. Less well characterized is the impact these two diseases have upon each other during times when patients are asymptomatic. At our site, disease prevalence estimates for both HIV (7-30%) and malaria (0-40%) are high and vary greatly with both immediate neighborhood and, for malaria at least, the methodology used for detection. Under an ethically approved protocol, we are collecting filter paper blood spots at the time of donations during blood drives across Nyanza Province. Reoptimized real-time RT-PCR assays have been developed to use as an efficient and highly sensitive way to detect any malaria, and subsequently falciparum gametocytes, in dried patient blood. Routine ELISA-based HIV screening data from normal blood bank procedures are used to detect HIV status. Prevalence and disease interaction data from this study will support future work defining the impact of HIV and associated clinical prevention therapies like trimethoprime/sufamethoxasole prophylaxis on malaria transmission in this holoendemic setting.

545

COMPARING AND VALIDATING MATHEMATICAL MODELS OF MALARIA TRANSMISSION USING BAYESIAN METHODS

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Mathematical models were used in previous eradication campaigns to predict the expected rates of transmission decline. However, discrepancies between model predictions and outcomes seriously compromised the campaigns. We have applied rigorous statistical methods to account for, and express, model uncertainties. We fitted models for malaria transmission dynamics to multiple datasets simultaneously, including age-stratified parasite prevalence measured by microscopy and in some cases PCR across a wide range of EIRs, and the age-stratified incidence of clinical disease from 2 EIR settings. We used Bayesian methods to incorporate additional information on parameters that cannot be readily identified from these data, such as the infectivity of humans to mosquitoes from feeding studies of mosquitoes on humans, and to formally compare different model structures. The best-fitting model included the development of immunity against acute clinical malaria and blood-stage parasites dependent on both age and force of infection, age-dependent heterogeneity in exposure and super-infection. The model reproduced well the patterns seen across different ages and transmission intensities. The ability of the methods to account for both model and parameter uncertainty by expressing predictions with uncertainty bounds will be demonstrated graphically. In conclusion, Bayesian methods allow us to incorporate prior knowledge of biological parameters when fitting models to epidemiological outcome data in a single coherent framework. They also provide a natural method to quantify the uncertainty in model predictions. Given the lessons from model use in the previous eradication campaigns, the latter is essential to avoid failure of the intervention program or misinterpretation of preliminary results from such programs.

546

SUCCESSFUL INTRODUCTION OF ARTESUNATE AND AMODIAQUINE IS NOT ENOUGH TO FIGHT MALARIA - RESULTS FROM AN ADHERENCE STUDY IN SIERRA LEONE

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Malaria diagnosis and, treatment are offered free of charge by Médecins Sans Frontières (MSF) to 150 000 people in eastern Sierra Leone. Since 2004, the first-line drug combination has been artesunate and amodiaquine (AS+AQ), but to be effective, it must be taken according to correct protocol. We aimed to measure adherence to AS+AQ in patients treated for uncomplicated malaria. We included patients ≥ 1 year old who received AS+AQ in MSF community health centres (CHCs) after confirmed diagnosis of uncomplicated falciparum malaria. Adherence was measured by home visits the day after the last treatment dose. Patients or carers were interviewed and remaining AS+AQ tablets were counted. In total, 118 patients were visited at home: 27 (22.9%, 95%CI 15.2-30.6) patients had ≥ 1 tablets left at the time of the visit and were defined as certainly non-adherent; 34 (28.8%, CI 20.5-37.1) were defined as probably non-adherent (verbal account of incorrect [n=27] or incomplete [n=7] intake); and 57 (48.3%, CI 39.2-57.5) as probably adherent. The main self-reported reasons for incomplete intake were sickness after one dose of AS+AQ (32%, 11/34), no food available for drug intake (15%, 5/34) and forgetting to take them (12%, 4/34). The main self-reported reasons for incorrect intake were vomiting after drug intake (45%, 12/27); 37% (10/27) said they were given incorrect instructions in the CHCs. 81% (46/57) of probably adherent patients said they followed the instructions given at the CHC. The results of this study suggest that adherence to treatment with AS+AQ by patients should not be taken for granted. Even a well established treatment programme should monitor adherence regularly. Only some of the factors affecting adherence can be addressed directly in an operational setting. Our study suggests that giving clear explanations on correct AS+AQ use should include discussion of disease symptoms as well as possible treatment side effects, and how to manage them. Other factors are more difficult to influence, such as patients forgetting to take their treatment dose.

547

MALARIA PREVALENCE IN TSUNAMI-AFFECTED DISTRICTS OF ACEH, INDONESIA

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Malaria is endemic to Indonesia. There is little prevalence data available from Aceh Province, however, due to the long-standing separatist conflict in the province and because surveillance systems were compromised by decentralization of the public health system. The MENTOR Initiative, which specializes in malaria control in humanitarian emergencies, was one of the non-governmental organizations to respond to the 2004 Indian Ocean Tsunami in Aceh. Data on malaria prevalence were gathered to guide and evaluate programmatic efforts. The MENTOR Initiative conducted community-based malaria prevalence surveys in 2005 and 2006 in five districts along the tsunami-affected western coastline. Individuals in randomly selected households were consented for testing using blood smears. Positive cases were treated according to guidelines. 11,763 individuals in 3771 households were tested. The overall slide positivity rate for *Plasmodium* spp. was 2.2%. Slide positivity rates ranged from 0 to 55% among villages. Overall, 57% of the 262 cases were infected with *P. falciparum*, while 43% were infected with *P. vivax*. A majority of affected patients were male, and over the age of 10 years. In conclusion, local prevalence data is needed to design effective community-based malaria control programs, as endemnicity varies greatly within districts. Certain villages were found to be hyperendemic, with slide positivity rates far higher than average in Indonesia, and similar to Sub-Saharan Africa. There is a need for ongoing malaria surveillance in Aceh Province to monitor prevention and treatment efforts.

548

ESTIMATES OF MALARIA AT COMMUNITY LEVEL THROUGH COMMUNITY-OWNED RESOURCE PERSONS (CORPS) STRATEGY BY EARLY DIAGNOSIS AND TREATMENT OF FEVER CASES IN NORTH-EASTERN TANZANIA

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Early diagnosis and treatment has been advocated as best strategy for malaria case management. Community owned resource persons (CORPs) were employed to provide such services at community level. The main aim of the study was to use CORPs strategy to estimate the burden of malaria in preparation of a site for malaria vaccine trial. The data reported here is for February 2006 to December 2008 period in 4 villages with differing malaria transmission. Passive case detection (PCD) of fever was through CORPs. Cases consulting CORPs had morbidity questionnaire completed and axillary temperature taken. Blood smear was taken in 2006 while both BS and rapid diagnostic test (RDT) was taken during 2007-08 period. Sulfadoxine/pyrimethamine (SP) was used as first line antimalarial in 2006 whilst Artemether-lumefantrine (ALu) was introduced from February 2007. Underfives were treated according to IMCI guidelines irrespective of RDT results. 11,038 cases were attended during 35 months period with 2365(21%) being underfives. Around 30.8% of all cases had measured fever (≥37.5). Overall, 41.5% of fever cases were positive for malaria parasites. Logistic regression, adjusting for strata showed malaria cases in 2007 was 2.26-fold compared to 2006 (OR =2.262,95%CI:2.032,2.52) and for 2008 it was 13% higher than 2006 (OR=1.13,95%CI=1.01,1.27). Clinical malaria (fever ≥ 37.5°C plus *P. falciparum* ≥ 2500 rings/µl) was, 9.5% across three years (2006: 6.4%, 2007:13.1%, and 2008: 8.6%). Overall incidence of malaria was 42/1000 person-years at risk in 2006 during SP use compared to 115/1000 person-year at risk during ALu use; giving a 2.7 fold increase. Observed increase in malaria cases in 2007 and the higher incidence in ALu era might be due to increased rainfall and poor compliance. Use of RDT during ALu era helped reduce over-treatment in adults. Our data has provided information on malaria burden in the study area which in combination with DSS data, were utilized to select one village for testing MSP3 malaria vaccine.

549

MALARIA IN THE FIRST YEARS OF LIFE AT A TIME OF BEDNET USE AND ARTESUNATE COMBINATION THERAPY IN THE KASSENA-NANKANA DISTRICT OF NORTHERN GHANA

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A large (N=2279) birth cohort study that commenced in March, 2006 may tell us if increasing use of bednets and a national policy change in 2005 making AS-AQ standard treatment for uncomplicated malaria is slowing or reducing malaria morbidity and mortality among young children in Northern Ghana. During the first 32 months of study 94% of the enrolled children were brought to hospital or health clinic for diagnosis and treatment. Slide proven malaria accounted for 31% of the 16504 outpatient visits made (Ave. 7.7/child; range: 1-29) and was detected in 1708 of 2279 (79%) children. Bednet use was professed in 88% of these cases, fever (>37.5) was measured in 61%, mean Hb was 8.8 g/dL, and 26% of parasitemias were >20,000/µL. Severe anemia characterized 4.4% of cases and 1.2% fit the WHO classification of severe malaria anemia. All received AS-AQ treatment. Among 3362 secondary or repeat outpatient visits with slide-confirmed malaria, 94% occurred more than 14 days after treatment of the primary case and may represent true re-infections. Annualized incidence of primary symptomatic malaria was 28% overall and by sector of residence ranged from 13 (town) - 31.5% (rural). Mean age of primary infection was 11.6 mos. overall, and by sector ranged from 10.4 (rural) to 14.5 (town) mos. Mean Hb was comparable in all sectors at the time of the primary infection, suggesting no underlying nutritional differences. Malaria was diagnosed in 54% (442/818) of children who were hospitalized and accounted for 565 of 1327 (43%) admissions. Mean age of hospitalized malaria cases was 17.4 mos.(95% CI: 16.8-18.0), mean Hb was 8.0 g/dL (95% CI: 7.8-8.2), 17% had Hb <6.0, and 34% had parasitemias >20,000/µL. Only 5.1% of these cases fit the WHO definition of severe malaria anemia. Among 376 admitted children with no detectable parasitemia, mean age was 12.5 (95% CI: 12.0-13.0), mean Hb was 9.7 (95% CI: 9.5-9.8), and 7% had Hb <6.0 g/dL. Case fatality rate proximal to date of admission in children with malaria was 3.4% (15/442) compared with 6.4% (24/376) in those with no detectable parasitemia.

550

MALARIA INFECTION IN INDIVIDUALS TAKING MEFLOQUINE DOES NOT INDUCE ANTIBODY RESPONSE TO MSP1$_{42}$

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A sensitive biomarker of malaria infection would allow for phase 3 clinical trials to prove efficacy of prophylactic drugs using active controls rather than placebo. Prior studies had suggested that individuals taking suppressive prophylaxis with mefloquine (MQ) develop antibodies to the blood-stage antigen MSP1$_{42}$. To determine the sensitivity of this marker for identifying malaria infection in individuals taking mefloquine we conducted a human *Plasmodium falciparum* challenge study. 23 volunteers received a loading dose of 250mg of MQ daily for 3 days then once weekly for four weeks. 6 individuals were enrolled as infectivity controls to ensure that mosquitoes used in the challenge were infectious. At the completion of MQ loading dose, all individuals underwent

sporozoite challenge. $MSP1_{42}$ IgG ELISA was performed on serum taken from baseline and serially collected over 6 months. Seroconversion was defined as a four-fold rise in titer. All 6 infectivity controls developed detectable parasitemia (5/6 developed fever, 1 remained asymptomatic). No volunteer in the MQ cohort developed detectable parasitemia or symptoms consistent with malaria by the end of 6 month follow up. At day 84 after challenge, 4/6 infectivity controls had a four-fold rise in anti-$MSP1_{42}$ IgG. Median time to seroconversion was 42 days (range 21 to 56 days). Mean fold-change in antibody titer among seroconverters at day 84 was 17. No member of the MQ cohort seroconverted at any time point. In concllusion, ahough anti-$MSP1_{42}$ ELISA appears to be a sensitive method of detecting recent infection with *P. falciparum* in individuals treated very early after patent parasitemia, individuals receiving suppressive prophylaxis with MQ do not generate antibodies to this blood-stage antigen. Though this antibody appears not be a useful marker in an active control drug trial, it may be useful in confirmation of malaria in individuals treated empirically. It should also be predictive of on-going malaria transmission in persons not taking prophylaxis.

551

ANALYSES OF CD8⁺ T CELL IMMUNE RESPONSES DURING THE *PLASMODIUM YOELII* BLOOD STAGE INFECTION

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It is well known that $CD8^+$ T cells are critical for conferring hosts' protective immune responses against the liver stage of malaria. On the contrary, the lack of MHC molecule on red blood cells has questioned any roles of $CD8^+$ T cells against its blood stage infection. This was supported by an observation that the depletion of $CD8^+$ T cells during the blood stage infection did not affect its natural course and also its outcome. However, since there are still limited analyses of $CD8^+$ T cell immune responses during the blood stage infection, their roles in hosts' protective immune responses have not been thoroughly elucidated. In addition, even if they are immunologically dispensable during the natural course of infection, it does not necessarily mean that the active induction of antigen-specific $CD8^+$ T cells doest not function to contain the expansion of blood stage malaria. To address the question whether the $CD8^+$ T cells are indispensable 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 well-defined H-$2K^b$-restricted, $CD8^+$ T cell-inducing epitope, ANYNFTLV. The epitope was first identified on a *Trypanosoma cruzi* antigen as an epitope which could confer $CD8^+$ T cell-dependent protective immunity. Expression of the epitope by the transgenic malaria 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 parasite. We have then elucidated the efficacy of prime/boost recombinant virus vector vaccination, the most effective vaccination protocol for the induction of maximal number of ANYNFTLV-specific $CD8^+$ T cells, against the infection with ANYNFTLV-expressing transgenic malaria. The critical roles of $CD8^+$ T cells during the malarial blood stage infection and their background immunological mechanisms will be discussed.

552

ANTIBODY LEVELS TO AMA1 AND MSP142 IN MALIAN INFANTS

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Infants in Bancoumana, Mali, were followed through malaria transmission seasons to determine baseline parameters prior to clinical trials of blood stage malaria vaccines. One hundred-five infants age 6-24 weeks were enrolled in August 2007 and followed during the transmission season until January 2008. Infants were seen monthly and at unscheduled visits. Anti-AMA1 and MSP142 antibody levels were obtained at enrollment and at the end of follow up for each infant. Of the infants enrolled, 75.2% and 24.8% had a detectable level of antibody to AMA1 and MSP1 at enrollment respectively. At the end of the transmission season the proportion of infants with detectable antibody to AMA1 had decreased to 20.4% while the number of infants with detectable antibody to MSP1 had increased to 38.8%. Of the 98 infants followed through the season, 42 (43%) had at least one episode of asexual falciparum parasitemia detected by smear at either scheduled or unscheduled visits. Having an episode of parasitemia detected during the transmission season was significantly associated with an increase in anti-MSP1 antibody but not with anti-AMA1 antibody (WMW; $p<0.00001$, $p=0.49$ respectively). Previous studies of adults in Mali show that anti-AMA1 antibody levels are generally higher than anti-MSP1. Maternal antibody may account for the higher levels of anti-AMA1 antibody in infants at the start of the transmission season. In this population, malaria infection in the first year of life does not increase anti-AMA1 antibody levels, but does increase anti-MSP1 antibody. This may have implications for vaccination of infants with blood stage malaria vaccines based on these antigens. Data from the second cohort (enrolled in 2008) will also be presented.

553

DUFFY ANTIGEN RECEPTOR FOR CHEMOKINES INFLUENCES LEUKOCYTE POPULATIONS AND CIRCULATING INFLAMMATORY MEDIATORS IN KENYAN CHILDREN WITH MALARIA AND HIV-1

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The Duffy antigen receptor for chemokines (DARC) is known for its ability to confer resistance to *P. vivax* infection when absent from RBC membranes. Recently, data from studies in African Americans suggest that -67CC (rs2814778) increases susceptibility to HIV-1 acquisition, but slows progression to AIDS. Our goal was to determine if variation in DARC C-67T plays a role in HIV outcomes and circulating inflammatory mediators. Children (n=679) were stratified into three groups [HIV-1(-), HIV-1 exposed (exp), and HIV-1(+)], and genotypes were determined with the ABI TaqMan allelic discrimination assay (C_15769614_10). All children were recruited at the Siaya District Hospital, western Kenya, during their first febrile visit. Complete hematological and monocytic/neutrophilic hemozoin profiles were also determined. Circulating inflammatory mediators were determined with the BioSource hu-Cytokine 25-Plex assay. Statistical significance of parametric (ANOVA, Student t-test) and

non-parametric (Kruskal-Wallis, Mann-Whitney U, Chi-square) tests was set at P<0.05. Distribution of genotypes in the overall population was 642 CC, 20 CT, and 17 TT. Genotypic distribution among the three HIV classifications was: HIV-1(-) 73.2% CC, 80.0% CT, 76.5% TT; HIV-1(exp) 21.5% CC, 15.0% CT, 23.5% TT; and HIV-1(+), 5.3% CC, 5.0% CT, 0% TT. There was significant departure from Hardy-Weinberg expectations in the overall population and among the HIV categories. Genotypic distributions were not significantly different across the HIV groups (P=0.833). In the overall population, white blood cell (P=0.043), monocyte (P=0.025), and lymphocyte (P=0.044) counts were lowest among the TT group relative to CC individuals. Grouping of HIV-1(+) and HIV-1(exp) individuals according to the T allele revealed significantly lower IFN-α (P=0.035), IL-2 (P=0.053), IL-2:IL2R ratio (P=0.048), and GM-CSF (P=0.022) relative to the CC genotype. Conversely, those with the T allele had ~100-fold higher circulating RANTES than the CC genotype (P=0.071). Although the T allele was rare in this population, results here suggest that the DARC polymorphism may play a role in conditioning the outcomes to HIV-1, and possibly malaria, since polymorphic variability influences circulating inflammatory mediator concentrations important in these diseases.

554

EVALUATION OF CYTOKINE LEVELS AND DISEASE SEVERITY IN *PLASMODIUM FALCIPARUM* MALARIA PATIENTS FROM THE PERUVIAN AMAZON BASIN

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Plasmodium falciparum causes the most severe form of malaria and can potentially lead to death. The development of disease pathology and resolution of infection depends on a balanced and timely immune response, which involves the production of antibodies and cytokines. Pro-inflammatory cytokines are associated with faster parasite clearance but also can lead to an increased risk of mortality, while anti-inflammatory cytokines may prevent severe clinical outcomes. We aimed to investigate plasma cytokine levels in patients with falciparum malaria from a hypoendemic area, the Peruvian Amazon basin. We obtained acute sera from 27 patients presenting with severe malaria symptoms between 1998 and 1999, and 37 patients with uncomplicated malaria in 2006. Pro-inflammatory (IL-2, IL-12, IFN-γ and TNF-α) and anti-inflammatory (IL-4 and IL-10) cytokines in plasma samples were measured by a multiplex assay using the Bio-plex™ system. The concentrations of IL-10, IL-4, IFN-γ and TNF-α were significantly higher in plasma from the severe malaria patients than from the uncomplicated malaria patients (Mann-Whitney's test, $p < 0.01$). In contrast, there were many patients with IL-2 and IL-12 concentrations below the detection limit of the assay. Non parametric correlations, assessed by the Spearman's rank test, showed that IL-10 is positively correlated with IFN-γ and IL-4 in severe malaria patients. In both patient groups, parasitemia and TNF-α are positively correlated with IL-10 and IL-4, and there is also a positive correlation between TNF- α and IFN- γ. Elevated levels of IL-10 in severe malaria patients and its positive correlation with high parasitemia suggest that in very high concentrations IL-10 may have a detrimental rather than protective effect on disease severity.

555

A VARIANT WITHIN THE STEM CELL GROWTH FACTOR (SCGF) PROMOTER (-539C/T) IS ASSOCIATED WITH PROTECTION AGAINST PEDIATRIC SEVERE MALARIAL ANEMIA AND FUNCTIONAL CHANGES IN CIRCULATING SCGF

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Plasmodium falciparum malaria is one of the leading global causes of infectious disease burden. In holoendemic *P. falciparum* transmission areas, such as western Kenya, severe malarial anemia (SMA) results in high rates of pediatric morbidity and mortality. Although the patho-physiological basis of SMA (Hb<6g/dL) remains unclear, we recently demonstrated that suppression of a novel hematopoietic growth factor that is important for promoting erythroid and myeloid colony development, stem cell growth factor [SCGF, C-type lectin domain family member 11A (CLEC11A)], is associated with enhanced development of SMA and a reduced erythropoietic response. To extend these investigations, the relationship between a novel SCGF promoter variant (-539C/T, rs7246355) and susceptibility to SMA was investigated in children (n=486) with falciparum malaria from western Kenya, a holoendemic *P. falciparum* transmission area. Hematological and parasitological profiles were determined in all study participants. SCGF -539C/T genotypes were determined using a Taqman 5´-allelic discrimination assay. Circulating SCGF levels were determined using the enzyme-linked immunosorbent assay. Frequencies of the -539CC, CT and TT were 27.5%, 36.8%, and 35.7%, respectively. Multivariate logistic regression analyses controlling for potential confounders demonstrated that homozygous T (-539TT) individuals were protected against SMA (OR; 0.59, 95% CI, 0.37-0.96; *P*=0.034) relative to CC (wild type) carriers. In addition, carriers of the TT genotype had significantly higher circulating (*P*=0.018) and PBMC culture supernatant (*P*=0.041) SCGF levels than individuals with the CC genotype. The results presented here demonstrate that variation in the SCGF promoter is associated with elevated SCGF production that is associated with protection against SMA.

556

INTERLEUKIN-23 RECEPTOR POLYMORPHISM (C/T) IS ASSOCIATED WITH PROTECTION AGAINST SEVERE MALARIAL ANEMIA IN KENYAN CHILDREN

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The IL-12/IL-23 signaling pathway is important for mediating clinical outcomes in infectious and inflammatory diseases. Recent studies have implicated IL-23 in autoimmune inflammatory diseases while children with severe malarial anemia (SMA) were reported to have elevated IL-23 plasma levels. Although polymorphisms within the IL-23 receptor (IL-23R) have been associated with several autoimmune diseases, their role in regulating malaria disease outcomes remains largely unexplored, particularly in *Plasmodium falciparum* holoendemic transmission areas. We therefore investigated the impact of IL-23R (rs7530511) variants in conditioning SMA (Hb<5.0 g/dL, any density parasitemia). Children (aged 3-36 mos.; *n*=486) presenting at the Siaya District Hospital, western

Kenya, a *P. falciparum* holoendemic transmission area were enrolled in the study. Complete hematological, parasitological, and clinical indices were determined. Genotyping of IL-23R C/T was performed by Taqman 5'-allelic discrimination assay. Genotypic prevalence was CC (66.9%), CT (27.6%) and TT (5.5%), with allele frequencies of C=0.81 and T=0.19, respectively. In a multivariate logistic regression model controlling for potential confounding effects of age, gender, sickle-cell trait, HIV and bacteremia status, individuals homozygous for the T allele were 63% less likely to develop SMA (OR; 0.37, 95% CI 0.14-0.95, *P*=0.038) relative to the wild-type individuals (CC). These results suggest that variation in the IL-23R C/T may be involved in conditioning SMA in children exposed to holoendemic *P. falciparum* transmission.

557

ASSOCIATION BETWEEN MIP-1A (MIP-1A) PROMOTER HAPLOTYPES AND HIGH-DENSITY PARASITEMIA IN CHILDREN FROM WESTERN KENYA

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Macrophage inflammatory protein (MIP)-lα (CCL-3) is an important inflammatory and hematopoietic mediator. In malaria, MIP-1α is elevated in children with overlapping hyper-parasitemia and mild-to-moderate anemia. A biallelic dinucleotide repeat [-906$(TA)_{2-6}$], and single nucleotide polymorphisms (-3094A/T and -6971C/T) located in the MIP-1α promoter have been associated with several autoimmune diseases and HIV-1, but their roles in conditioning severe malaria have not been determined. We, therefore, investigated the influence of MIP-1α haplotypes [-906$(TA)_n$/-3094A/T/-6971C/T] on severe malarial anemia (SMA, Hb<6.0g/dL, with any density parasitemia), high-density parasitemia [HDP, *P. falciparum* (*Pf*) parasites ≥10,000/μL], and MIP-1α production in children with *Pf* malaria (*n*=350) from Siaya District, western Kenya. Genotyping of the -906 polymorphism was performed using PCR and alleles were determined by the GeneMapper software, while -3094 and -6971 genotypes were determined using the Taqman 5' allelic discrimination assay. Circulating MIP-1α levels were measured as part of a 25-plex cytokine assay. The major haplotypes in the cohort were: $(TA)_2$AT (30.9%), $(TA)_4$AT (61.4%), $(TA)_4$TC (22.6%), $(TA)_4$AC (20.9%), and $(TA)_5$TC (22.9%). No significant association between MIP-1α haplotypes and SMA was observed. Multivariate logistic modelling revealed that the [$(TA)_2$AT] haplotype was associated with increased risk of HDP (OR; 1.79, 95% CI; 1.01-3.18, *P*=0.047). In addition, median (Q1-Q3) parasite density/μL [42,244 (15,491-86,376) vs. 26,213 (8,459-69,189), *P*=0.059] and plasma MIP-1α levels (pg/mL) [109.4 (78.3-160.3) vs. 127.3 (89.1-197.3), *P*=0.043] were lower in the [$(TA)_2$AT] carriers relative to non-[$(TA)_2$AT] carriers. These results illustrate that the [$(TA)_2$AT] haplotype is a genetic risk factor for development of HDP, at least in part, through decreased MIP-1α production.

558

SEROPREVALENCE OF IGG ANTIBODIES TO *PLASMODIUM VIVAX* MSP-1 ANTIGEN AND *PLASMODIUM FALCIPARUM* GLURP R2 ANTIGEN IN THE AMAZON AREA, IQUITOS-PERU

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Naturally acquired antibodies to Merozoite Surface Protein-1 (MSP-1) and Glutamate Rich protein R2 (GLURP R2), antigens that are tested in this study as targets of immunity to *Plasmodium vivax* and *P. falciparum* respectively, were measured in a survey of peri-urban communities in Iquitos, Loreto. We evaluated total IgG response to MSP-1 and GLURP R2 antigens in 345 individuals positive to *P. vivax* or *P. falciparum* by microscopy and PCR in order to evaluate the seroprevalence to both antigens in this area. Recombinant MSP-1 and GLURP R2 antigens were used in indirect ELISA assay for total IgG that was performed from dried blood spots on filter paper treated with PBS Blotto in order to elute the sample. The plates were read at absorbance of 405 nm. 24.1% (n=83 subjects) had IgG positive response to MSP-1 antigen and 11.3% (n=39 subjects) had IgG positive response to GLURP R2 antigen. The IgG antibodies to GLURP R2 antigen were less prevalent compared to the response against *P. vivax* MSP- 1 in this study area. Moreover, 53 out of 83 subjects with IgG positive response to MSP-1 were *P. vivax* positive and 30 patients showed a negative diagnostic by PCR. On the other hand, we found that from the total number of individuals with IgG positive response to GLURP R2, 8 individuals were *P. falciparum*, 11 *P. vivax*, 18 negatives and 1 mix infection. In conclusion, the relationship between the positivity or high IgG response to the antigens tested in this research and the PCR specie-specific diagnosis found, could be correlated to cumulative malaria exposure or acquired immunity (presence of specific antibodies matching the MSP-1 antigen in infecting parasites) and the prevalence of *P. vivax* in this area.

559

PLASMODIUM FALCIPARUM MEROZOITE SURFACE PROTEIN 6: GENETIC DIVERSITY AND ANTIBODY RESPONSES IN A LONGITUDINAL COHORT STUDY IN THE PERUVIAN AMAZON

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Plasmodium falciparum Merozoite Surface Protein 6 (PfMSP6) forms part of a multi-protein complex on the *P. falciparum* merozoite surface, along with PfMSP7 and the major blood stage vaccine candidate, PfMSP1. Given that PfMSP1 is the most abundant protein component of the merozoite surface, PfMSP6 is by association an antigen of considerable interest for vaccine development. However, field studies of PfMSP6 have been limited, and it has only recently been appreciated that PfMSP6 is, like numerous other merozoite surface proteins, a dimorphic antigen. We have used samples from an ongoing longitudinal epidemiological cohort study in the Peruvian Amazon to carry out a systematic study of both PfMSP6 genetic diversity and the antibody responses that PfMSP6 generates during *P. falciparum* infection. *P. falciparum* transmission at the study site is hypoendemic, with less than one infection per person per year, meaning that individuals are usually infected with clonal *P. falciparum* infections spaced many months apart. We have genotyped PfMSP6 in more than 400 *P. falciparum* infections spanning four transmission seasons, and established that both PfMSP6 alleles are circulating at the study site, with statistically significant changes in allele frequency between transmission seasons. We have also performed ELISA assays using two different PfMSP6 N-terminal antigens, based on the genotypes currently circulating at the

study site, as well as a PfMSP6 C-terminal antigen that is conserved in both alleles. Our genotyping data established that all infections consisted of a single PfMSP6 allele, so we were able to compare the immune response against the currently infecting PfMSP6 allele with the response against a PfMSP6 allele that the individual is not currently exposed to, nor, given the transmission dynamics, have they been on average for at least a year. Responses against PfMSP6 N-terminal antigens were generally low, with the majority of individuals falling below the negative cut-off, but there was little quantitative difference between responses to the infecting and non-infecting allele, suggesting some measure of cross-reaction. We are also isotyping and measuring the longevity of antibody responses against each antigen. This systematic study of anti-PfMSP6 domain-specific antibody responses has important implications for the viability of PfMSP6 as a vaccine target.

560

A NOVEL APPROACH TO DESIGN MULTICOMPONENT BLOOD STAGE MALARIA VACCINES

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The development of an effective malaria vaccine would contribute to reduce the disease burden. There is evidence that IgG antibodies mediate naturally acquired immunity to blood stage malaria, and the antibody dependent cellular inhibition (ADCI) was identified as a mechanism by which antibodies control the parasite densities. Merozoites, the invasive form of the asexual parasite, and released antigens, appear as the main targets of protective antibodies in endemic areas. Combining carefully chosen regions from merozoite proteins was chosen as an approach to design improved vaccine combinations. Detailed antigenic analyses were conducted using recombinant proteins and synthetic peptides to characterize the antibody responses in sera from malaria immune Africans. The biological anti-parasite activity of human antibodies specific to each antigen and those induced by immunisation of rodents were evaluated in ADCI functional assays. The genetic polymorphism was evaluated by nested PCR and sequencing of the selected regions with DNA specimens from malaria patients isolates. Antibodies to the selected merozoite antigens harboured by individuals naturally exposed to malaria were of IgG1 and IgG3 cytophilic subclasses, the latter being predominant for some of them such as MSP3 family antigens or MSP1 block 2. The synthetic peptides were antigenic, defining B cell epitopes mimicking native parasite ones. Most of the corresponding human affinity-purified specific antibodies were able to inhibit *P. falciparum* growth in cooperation with blood monocytes in ADCI assays. In some cases the ADCI effect was as strong as that of IgG from protected African adults. A first polyantigenic construction combining cross-reactive regions from the MSP3 family of proteins yielded in mice, specific antibodies with high avidity and functional activity. This approach represents a substantial improvement for selecting antigens to include in multi-component blood stage *falciparum* vaccines, by assessing first their individual efficacy before combing them.

561

STABILITY OF THE *PLASMODIUM FALCIPARUM* AMA1 VACCINE FORMULATED IN MONTANIDE ISA 720

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Plasmodium falciparum apical membrane antigen 1 (AMA1) is an asexual, blood-stage vaccine candidate against the malaria parasite. The AMA1-C1/ISA720 vaccine is designated to represent the mixture of AMA1-FVO and AMA1-3D7 at a 1:1 ratio formulated in Montanide ISA 720. With the goal to develop an AMA1-C1/ISA720 vaccine against malaria, it is important to determine the stability of this formulation which may require extended storage time. In this study, AMA1-C1 in saline containing 50 mM glycine was formulated in Montanide ISA 720 at 10 and 40 µg/ml. Stability of AMA1-C1/ISA720 at different time points (0, 5, 12 or 18 month) was studied by determining the mean particle size (diameter of the mean droplet volume) with a Malven Mastersizer 2000 using laser diffraction, total protein content with modified Lowry assay and ELISA using His_5-tag antibody (for 18 month samples only), identity with western blot and integrity with SDS-PAGE/silver staining. Our results showed that the mean particle size of these emulsions increased over time, ranging from 0.77 µm at time 0 to 1.26 µm after 18 months of storage at 4°C. The SDS-PAGE/silver staining and western blot analyses demonstrated that the concentrations of AMA1-C1 at both doses also decreased over storage time when compared to those of the freshly prepared formulations. For the 10 µg/ml dose at the 18-month time point, the protein content was only 4.9 ± 2.6 µg/ml as detected by ELISA but was undetectable by modified Lowry assay (at the lower end of the detection range). For the 40 µg/ml dose at the same time point, the protein content was 42 ± 2.6 µg/ml by modified Lowry assay, but was only 7.9 ± 1.3 µg/ml by ELISA. Although the degraded protein fragments may still be present as shown by the modified Lowry assay, the results suggest that the integrity of the epitopes detected by mAbs (western blot and ELISA) were affected by long-term storage, resulting in significant loss in AMA1-C1 detected. The results of the present study indicate that the AMA1-C1/ISA720 emulsion was unstable and AMA1-C1 may be partially degraded after 18 months of storage.

562

IMMUNIZATION WITH N-TERMINAL REGION OF A GAMETOCYTE PROTEIN PFS230 SUCCESSFULLY INDUCE TRANSMISSION-BLOCKING ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM*

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The aim of the malaria transmission-blocking vaccine (TBV) is to block the development of malaria parasites in the mosquito and prevent the following infection to the host. While a gametocyte/gamete surface protein Pfs230 is one of the TBV candidate molecules for more than a decade, the investigation of Pfs230 as TBV candidate was significantly hampered by the difficulty of producing the correctly folded recombinant protein because of a large number of cysteine residues. In our previous study, we reported the success in producing recombinant Pfs230 proteins using wheat germ cell-free expression system and the antibody against Pfs230C, a portion of Pfs230 containing disulfide bond-constrained domains I-III as predicted by Gerloff et al. (2005) and its N-terminal disulfide bond-free pro-domain, effectively induced transmission-blocking activity in the presence of complements. In this study we focused on Pfs230C, and produced three C-terminal-truncated recombinant Pfs230C corresponding cysteine motif domains I-II (Pfs230C2), domains I (Pfs230C1), and N-terminal disulfide bond-free pro-domain alone (Pfs230C0) using the cell-free system. Rabbit antisera against these recombinant proteins were generated by immunization with Freund adjuvant. All antisera reacted on the surface of cultured *Plasmodium falciparum* NF54 gametocytes and gametes. Western blot analyses using cultured gametocytes revealed that all the antisera recognized the 360-kDa form of parasite-produced Pfs230 and were conformation dependant. All the antisera reduced the infectivity of NF54 parasites to Anopheles stefensi mosquitoes by membrane feeding assay. Moreover the reduction efficacy was enhanced in the presence of complement. Our data suggests that the N-terminal pro-domain of Pfs230C is sufficient to induce the complement dependent transmission-blocking activity.

563

SHIFT IN EPITOPE DOMINANCE OF IGM AND IGG RESPONSES TO *PLASMODIUM FALCIPARUM* MSP1 BLOCK 4

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Plasmodium falciparum merozoite surface protein-1 (MSP1) has been extensively studied as a blood stage malaria vaccine candidate, with most work focused on the conserved 19 kDa and semi-conserved 42 kDa C-terminal regions and the hypervariable N-terminal repeat region. However, recent genotyping studies suggest that additional regions of MSP1 may be under selective pressure, including a locus of intragenic recombination designated as Block 4 within the 3' region of the gene. The current study examined the antibody response to the two parental and two recombinant forms of MSP1 Block 4 in study populations from Cameroon, Colombia, and Papua New Guinea differing in malaria transmission intensity, seasonality of transmission, and ethnic composition. Sera from all study populations contained IgM and IgG antibodies reactive with the parental and recombinant MSP1 Block 4 peptides. The MSP1 IgM response was co-dominant for Block 4 and C-terminal MSP1.42 (blocks 16-17) epitopes and cross-reactive against all four allelic Block 4 peptides. In contrast, the MSP1 IgG response was dominated by antibodies to the MSP1 42 kDa C-terminal polypeptide, with a lower seroprevalence of antibodies to MSP1 Block 4. The majority of IgG Block 4 antibodies were specific for individual Block 4 peptides. This pattern of recognition was consistently observed in all study populations. Subdominant epitopes have been found to be protective in several viral infections, and IgG antibodies directed against subdominant MSP1 Block 4 determinants may play a role in isolate-specific immunity to *P. falciparum*.

564

HOW WILL MALARIA EVOLVE IN RESPONSE TO A VACCINE?

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Drug resistance is one of the most medically relevant forms of pathogen evolution. To date, vaccines have not failed with the same depressing regularity as drugs. Does that then make vaccines evolution-proof? In the face of vaccination, pathogens are thought to evolve in two ways: by evolving epitope changes at the antigenic target of vaccination (epitope evolution); or, by evolving changes at other antigenic loci, some of which may involve virulence (virulence evolution). The fundamental difference between these two forms of evolution is that virulence evolution could lead to disease outcomes in unvaccinated people that are more severe than would have been seen prior to evolution. One of the theoretical assumptions of virulence evolution is that more virulent parasites will have a selective advantage over less virulent parasites in an immunized host, and are thus more likely to be transmitted. The assumption is that more virulent parasites may be competitively more superior in mixed infections, or may be better able to evade/modulate the host immune response. Thus, the aim of this work was to experimentally test whether more virulent parasites have a within-host selective advantage in an immunized host or whether vaccine efficacy is more likely to depend on genetic differences at the targeted sites of vaccination. We used clones (genotypes) of the rodent malaria *Plasmodium chabaudi* originally derived from wild-caught Thicket (Thamnomys rutilans) rats to infect laboratory mice and a rodent analogue of the candidate blood-stage malaria vaccine apical membrane antigen 1 (AMA-1). We found that when one *P. chabaudi* genotype was serially passaged through naïve mice, despite no sequence changes at the AMA-1 locus, the derived line was more virulent and was subsequently less well controlled by vaccine-induced immunity. Furthermore, in other experiments we found within host competition not to be immune-mediated. Thus our results suggest that vaccination has the potential to select for more virulent parasites but that the selective advantage is likely to be independent of competition. The selective advantage may be attributable to the enhanced immune evasion of more virulent parasites. These results contribute towards a growing body of evidence that vaccines have the potential to differently alter the within-host parasite dynamics of particular pathogen genotypes and that in some cases they may select for more virulent genotypes.

565

PURIFIED IGGS WHICH ARE OBTAINED FROM MALIAN CHILDREN AND WHICH DO NOT BIND TO APICAL MEMBRANE ANTIGEN 1(AMA1) INTERFERE WITH THE BIOLOGICAL ACTIVITY OF AMA1-SPECIFIC IGGS AS JUDGED BY THE *IN VITRO* GROWTH INHIBITION ASSAY

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In our previous study, from Malian adult total IgGs, we separated Apical Membrane Antigen 1 (AMA1)-specific IgGs (AMA1-IgG) and IgGs which do not bind to AMA1 (non-AMA1-IgG) by using AMA1 affinity chromatography. The non-AMA1-IgG interfered with the *in vitro* growth-inhibitory activity of the AMA1-IgG. We have also shown that the non-AMA1-IgG reduced the activity of anti-AMA1 IgG from US volunteers immunized with AMA1 vaccine. However, it was unclear whether such "interfering" antibodies exist in children or infants, who are the main target population of the AMA1 vaccine and who have less previous exposure to malaria. We conducted a Phase 2 clinical trial with AMA1 vaccine; a total of 300 children (2-3 years old) received either the AMA1 vaccine or the comparator on Days 0 and 28. To address the question, we purified total IgG from individual plasma on Day 0 and 42, and made 4 pools from Day 0 IgGs (from both AMA1 or comparator groups), 3 pools from Day 42 IgGs in the AMA1 group and 3 pools from Day 42 IgGs in the comparator group. We then separated AMA1-IgG from non-AMA1-IgGs for each pooled IgG. The children's AMA1-IgGs showed similar growth-inhibitory activity as AMA1-IgGs from US vaccinees and Malian adults when ELISA units were normalized. When the children's non-AMA1-IgGs were tested at 4 mg/mL in the well, they showed <13% inhibition. When 4 mg/mL of these non-AMA1 IgGs were mixed with total IgG from US vaccinees (the US total IgG showed 66% and 58% inhibition for 3D7 and FVO parasites), both Day 0 and Day 42 non-AMA1-IgG from the children showed an interference effect on growth-inhibitory activity (10-59% reduction for 3D7 parasites and 13-53% for FVO). Interestingly, non-AMA1-IgG from higher AMA1 titer pools showed a higher interference effect than non-AMA1-IgG from lower AMA1 titer pools (Spearman Rank test, r_s=0.854, p<0.0001). This interference effect should be considered when evaluating growth-inhibitory activity of IgGs from an AMA1 vaccine trial in children who live in malaria endemic areas.

566

ALLELE-SPECIFIC EFFICACY OF AN AMA-1-BASED MALARIA SUBUNIT VACCINE

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Antigenic diversity in malaria parasites may pose a major obstacle to the development of an effective malaria vaccine. To assess whether vaccination with a monovalent AMA-1-based malaria vaccine selects for alleles different from the vaccine allele among vaccinees, *ama-1*gene sequences will be assessed for *P. falciparum* infections experienced by 400 children randomized 1:1 to receive either the FMP2.1/AS02A malaria vaccine or rabies vaccine in a Phase 2 safety and efficacy trial conducted in Bandiagara, Mali. Blood samples were collected at baseline, at scheduled intervals during the one-year follow-up period, and during routine malaria diagnosis. Parasite DNA will be extracted from pre- and post-immunization samples and the *ama-1* gene will be amplified by PCR and subjected to direct DNA sequencing. Homology will be defined based on the entire AMA-1 ectodomain as well as by specific clusters of amino acids suggested to be involved with clinical immunity. The odds of being in the AMA-1 vaccine group will be measured by applying a proportional odds model with p-distances as the dependent variable. Genetic divergence from the 3D7 vaccine strain will be measured by comparing haplotype diversity and nucleotide diversity parameters of sequences obtained from the control vaccine group to those of AMA-1 vaccine group at baseline and after vaccination. DNA extractions have been completed and sequencing is underway. This assessment of allele-specific efficacy and identification of alleles resistant to the vaccine will help in the design of a more universally protective malaria vaccine.

567

OPTIMAL TARGETS IN THE SPOROZOITE LIFECYCLE FOR PRE-ERYTHROCYTIC MALARIA VACCINES

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There has recently been considerable success in the development of pre-erythrocytic candidate malaria vaccines. A notable effort has been RTS,S combined with adjuvant AS01E/02D which has demonstrated 53% efficacy against clinical malaria in children aged 1-4, and 65.2% efficacy against first infection in infants. However it is still unclear which stage of the pre-erythrocytic lifecycle this vaccine actually targets; sporozoites in the skin, blood, or sequestered in hepatocytes in the liver. Using a within-host model of sporozoites injected from a single infectious mosquito, and their interaction with the host immune system, we investigate the parasite's journey from skin to successful development in the liver and hence calculate the probability of infection per bite. The impact of antibodies targeted against the sporozoite (for example antibodies directed against the circumsporozoite-protein) is also modelled to investigate the development of pre-erythrocytic immunity. The impact of pre-erythrocytic vaccines is also be modelled to investigate the point in the sporozoite's lifecycle against which they're effective. In particular we highlight bottlenecks in the sporozoite's lifecycle which may be optimal targets for vaccines. An example of such a bottleneck is the experimental observation of adult volunteers vaccinated with RTS,S who developed blood-stage infection after a sole sporozoite made it through the liver stage.

568

EVALUATION OF POTENTIAL MALARIA VACCINE ANTIGENS IN *PLASMODIUM YOELII*/MOUSE MODEL

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There is a great need to identify new malaria vaccine antigens. RTS,S, a *Plasmodium falciparum* CSP-based vaccine, can protect humans against a *P. falciparum* challenge. However, RTS,S may not be efficacious enough to be an effective stand-alone vaccine for military use. Evaluation of other *P. falciparum* vaccines, including subunit protein preparations of AMA1, $MSP1_{42}$ and SSP2/TRAP, has not been encouraging. Therefore, we are looking for new vaccine antigens. The United States Military Malaria Vaccine Program has identified several immunogenic pre-erythrocytic *P. falciparum* proteins that are recognized by sera and/or PBMCs from human volunteers immunized with a *P. falciparum* irradiated sporozoite vaccine. We have cloned the *P. yoelii* ortholog of the genes encoding these proteins and generated DNA and poxvirus vectors that express the *P. yoelii* antigens. Protection studies evaluating the efficacy of these vaccine vectors suggest that at least one of the new vaccine antigens may be able to protect mice against a *P. yoelii* sporozoite challenge.

569

COMBINATION OF SEROLOGICALLY DISTINCT ADENOVIRAL VECTORS IN PRIME-BOOST SCHEDULE FOR MALARIA VACCINATION

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Optimal protection against malaria undeniably requires both antibody responses and IFN-γ producing $CD8^+$ and $CD4^+$ T cells. In order to achieve such immunity, the successful malaria vaccine must have a broad spectrum of immune system activation, which is unlikely to be achieved by a single component vaccine. Combination of different vaccines, that can complement each other in the type of immunity they induce, is a highly promising approach. Heterologous prime-boost vaccination schedules consisting of adenoviral vectors with other types of vaccines are shown to be highly efficient in inducing such complex immunity. We demonstrated in previous studies that heterologous prime-boost vaccinations using adenovectors serotype 35 (Ads35), encoding the circumsporozoite (CS) antigen or liver stage antigen-1 (LSA-1), and protein-based vaccines effectively enhances the T-cell response induced by the protein vaccinations without impairing the humoral response. Here we report on the immunogenicity of a heterologous prime-boost schedule that combines the Ad35.CS vector with a serologically distinct adenovector Ad5.CS in rhesus macaques, which was evaluated after establishing the potency in mice. We show that the heterologous Ad35.CS/Ad5.CS prime-boost regimen elicits a robust CS-specific humoral and IFN-γ T-cell immunity. The level of the antibody responses achieved in rhesus macaques was superior to the response measured in serum of adults from endemic malaria region. Overall, our results demonstrate that a combination of adenovectors of distinct serotypes efficiently induces a complex immune response, such as required for protection against malaria, in nonhuman primates, and they warrant further research as a pediatric malaria vaccine.

570

COMPARING PRIME-BOOST REGIMENS OF *PLASMODIUM VIVAX* CS PROTEIN, DNA, AND ADENOVIRAL (AD5) VACCINES FOR IMMUNOGENICITY IN MICE

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Data from previous studies using protein, DNA, and adenoviral (Ad) vectors have demonstrated that immune responses induced by vaccine regimens using a single platform can be improved by heterologous prime-boost approaches.. A vaccine construct encoding a chimeric *P. vivax* CS protein, designated VMP001, has been previously described. In this study we used this platform to evaluate the effect of homologous and heterologous prime-boost regimens using recombinant protein, plasmid DNA and Ad5 constructs on the immune response. Inbred C57/BL6 mice (n=10 per group) were injected at suboptimal doses on days 0 and 28 with VMP001, the recombinant PvCS protein(1.0 µg) adjuvanted with Montanide ISA 720, PvCSP plasmid DNA (100 µg), and Ad5PvCSP (1.0 x 10^8 viral particles) in homologous and heterologous regimens. Differences in serum antibody levels were monitored by ELISA at days 14, 42, and 70. A comparison of antibody titers at day 70 indicated that a regimen that utilizes Ad5PvCSP to prime and PvCSP protein to boost may be optimal for induction of humoral responses. Monitoring of cell mediated immunity using splenocytes from vaccinated mice at day 70 was conducted using ELISpot assays to detect the interferon-γ (IFN-γ) expressing T cells. Data indicated that any regimen that included a PvCSP protein component to prime or boost resulted in greater numbers of IFN- γ expressing T cells than those that did not, and a homologous regimen utilizing PvCSP protein may be optimal for induction of cell mediated immunity. Flow cytometry is being conducted to identify the T cell subsets secreting IFN-γ in these experiments.

571

USE OF PRIME-BOOST COMBINATIONS OF ATTENUATED SPOROZOITE AND SUBUNIT VACCINES TO INDUCE POTENT PROTECTION AGAINST SPOROZOITE CHALLENGE AND DEVELOP A SCREENING TOOL FOR PROTECTIVE NOVEL ANTIGENS

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Sterile immunity can be induced in animals and humans by immunization with radiation-attenuated Plasmodium sporozoites and this model has led to current efforts to develop *P. falciparum* radiation-attenuated sporozoites for human use. Limited clinical data suggest that boosting these protective responses by exposure to unattenuated sporozoites may induce even stronger, longer-lasting immunity. Subunit vaccines, although generally achieving a lower level of protection, can also be improved by prime-boost regimens, and may prove to be a more feasible method for boosting the protection afforded by radiation-attenuated sporozoites. In this study, we aimed to use subunit vaccines in prime-boost regimens to enhance the duration and potency of the protection afforded by a suboptimal dose of radiation-attenuated P. yoelii sporozoites (IrrPySpz). In preliminary studies, immunization with suboptimal doses of IrrPySpz followed by challenge at 2 weeks protected mice at a moderate level, 58% (7/12), which waned to 17% (2/12) at 7 weeks. Boosting the primed mice with PyCSP pDNA before challenge at 7 weeks slightly increased level of protection from 17 to 33% (4/12), while no protection was seen in the mice that received the pDNA alone. The boosting effect was more pronounced when the order of the prime-boost regimen was reversed: immunization with pDNA alone was not protective (0/12), while boosting with a suboptimal regimen of IrrPySpz before challenge at 7 weeks protected 83% (10/12). The reproducibility of these results, the mechanism of protection, and the magnitude and longevity of the protective immune response are currently being investigated. Additional experiments will evaluate the potency of PyCSP delivered as a recombinant adenovector or poxvector rather than pDNA for priming or boosting IrrPySPZ-induced immunity. Once a protective regimen involving attenuated sporozoites and subunit vaccines is established, the model can be used as a sensitive screening method for identifying protective Py orthologs of novel Pf antigens under consideration as vaccine candidates.

572

SITE CHARACTERIZATION FOR A MALARIA VACCINE TRIAL IN THE SAPONÉ HEALTH DISTRICT IN BURKINA FASO: THE PREVALENCE OF PARASITES THAT MIGHT INTERFERE WITH THE ASSESSMENTS OF VACCINE SAFETY AND EFFICACY

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Field evaluation of safety and efficacy of malaria vaccine candidates for malaria control requires a systematic evaluation of confounding factors that may affect vaccine efficacy. One commonly overlooked confounder is the presence of parasites in the target population. Studies conducted in Africa and Asia indicates that helminthes infection adversely affect the clinical outcome of malaria infection. This suggests that helminthes can influence the acquisition of immunity against malaria. Thus it is important that future malaria vaccine trials, in areas where co-infection between malaria and worms is common, document the prevalence and diversity of parasitic infections. We conducted a cross sectional survey in volunteers aged 2 to 45 years during malaria high transmission season in the Sapone health district. During the 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 the qualitative and the quantitative determination of helminthes and *Schistosoma hematobium*. The diagnosis of intestinal helminthes was done by Kato-Katz thick smear examination technique. 349 females and 357 males volunteers were enrolled. The mean age of the volunteers was 15.3± 12.4 years. From 706 stools examined, 99 (14.0%) had helminth or other intestinal infections. The main helminth infections were *Ankylostoma duodenale* (6.2%), *Ascaris lumbricoides* (1.8%), *Trichuris trichiuria* (0.8%), *Enterobius vermicularis* (0.2%). The others intestinal parasites were *Hymenolepis nana* and *Taenia* sp; 4.8% of the volunteers. The seroprevalence of *W. bancrofti* was 13.03% (92). *S. hematobium* infection was present in 13 (1.8%) of the study population. The prevalence of malaria infection was 53.1% (Geometric mean 993.34 parasites/µl) for *Plasmodium falciparum*; 3.8% for *P. ovalae* and 8.3% for the *P. malariae*. *P. falciparum* gametocyte carriage was 13.5% (28.19 gametocytes/ µl). The prevalence of malaria infection was similar in the intestinal parasite infected and non infected groups: 50.5% IC95% [40.56 - 60.46] vs 53.5% IC95% [49.57-54.49]. In conclusion, these data show diversity and intensity of parasitic infections in Saponé health district area, which can interfere in vaccine efficacy. This should be considered when designing future malaria vaccine trial.

573

SEASONAL VARIATION IN SPECIES COMPOSITION AND FREQUENCY OF INSECTICIDE RESISTANCE ALLELES (KDR AND ACE-1R) IN THE *ANOPHELES GAMBIAE* COMPLEX FROM AN IRRIGATED RICE FIELDS AREA IN WESTERN BURKINA FASO

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Monitoring of the spread of insecticide resistance in field vector populations is a prerequisite for the implementation of efficient and sustainable vector control strategies based on the use of insecticides. Screening for resistance alleles in *Anopheles gambiae* populations is facilitated by the availability of molecular diagnostics to detect major target-site mutations, such as knock-down resistance (kdr) and insensitive acetylcholinesterase (ace-1R). *Anopheles gambiae* mosquitoes were collected resting indoors in two villages within a rice cultivation area in western Burkina Faso, from January to December 2007. Specimens were identified to species and molecular form and their genotype at the kdr and ace-1 locus was determined using PCR and RFLP protocols. The M form was largely predominant in our samples and was present all year round in both villages. S-form mosquitoes gradually appeared during the rainy season in the village at the margins of the rice fields (VK7) whereas it was very rare in the center of the rice cultivation area (VK5) throughout the survey. The frequency of both kdr and ace-1R mutations was higher in the S than in the M form at any time. In the M form, frequency of the kdr mutation was higher during the rainy season in both villages ($P<0.005$). We report occurrence of the ace-1R mutation in the M form, albeit at a low frequency (<1%). In conclusion, our results highlight the preoccupying status of insecticide resistance in *An. gambiae* populations from Burkina Faso, and suggest that comprehensive monitoring strategies need to consider population dynamics.

574

INFLUENCE OF INSECTICIDES RESISTANCE ON THE SALIVARY PROTEINS OF *CULEX PIPIENS QUINQUEFASCIATUS* MOSQUITO

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Culex quinquefasciatus mosquito has developed several resistant mechanisms to the main families of insecticides used in public health. Among these mechanisms, the insensitive acetyl cholinesterase (*Ace.1^R*) confers cross resistance to organophosphorous and carbamates. Fortunately, in an insecticide-free environment, this mutation is associated with a severe genetic cost that affects different biological systems. In insects, the saliva contains bioactive molecules (vasodilatators, anticlotting and anti-hemostatic proteins) which permit a successful blood meal and also facilitate pathogen transmission. In this context, we studied the differential expression of salivary proteins between susceptible and carbamate-resistant (*Ace.1^R*) strains of *Cx. quinquefasciatus* having a same genetic background. 2D-electrophoresis and SameSpots® software were used to determinate the variation of salivary proteins expression. The preliminary results showed that three majority saliva proteins of the D7 family have lower expression in the resistant strain compared to the susceptible strain. Conversely, proteins involved in metabolic reactions, were up regulated in the resistant strain. The results of an analysis including more replicats (n=17) will be presented. This differential expression according to the resistant status of the mosquito may have a repercussion on the biting behaviour and on the transmission of parasites/virus to vertebrate hosts. The next step will consist to study using a video based analysis system the feeding behaviour of susceptible and resistant mosquitoes in flying chambers. These studies will provide new elements to develop alternative insecticide resistance management strategies in *Culex* mosquito.

575

VARIABILITY IN MOSQUITO RESPONSE TO COMMERCIAL REPELLENT FORMULATIONS TESTED IN THE FOREST AREA OF CAMEROON

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Application of repellents to the skin is a common personal protection practice for preventing mosquito-borne diseases. Here, we tested the efficacy and persistence of three commercial repellent formulations against the bites of mosquito vector diseases. Four target doses (0.1mg/cm²; 0.3 mg/cm²; 0.6mg/cm² and 0.8mg/cm²) of each repellent ie 30%DEET (Buzz-Off™) and 25%IR3535 (Cinq-sur-Cinq™ and Prébutix™) or 90% ethanol as control were applied on the legs of volunteers who performed human landing catches to determine repellent efficacy. Effective dosages and persistence of each repellent were estimated by fitting a logistic plane model. During 48-days, 7,569 mosquitoes belonging to four genera were collected: *Mansonia spp* (67.3%), *Anopheles spp* (27.4%), *Aedes spp* (3.8%), and *Culex spp* (1.5%). After 8h exposure to mosquito bites, percentages of repellency provided by each of the three formulations were quite variable, ranging from 20 to 80%. Efficacy and persistence parameters were estimated only for *An. moucheti* and *Mansonia* spp. The effective dosages (ED_{50} and ED_{95}) as well as the effective half-lives obtained with the DEET-based repellent were highly variable among replicates in the case of *An. moucheti*. For *Mansonia* spp, the estimated ED_{50} value for the DEET-based repellent was ≈ 0.06 mg/cm². For the two IR3535-based repellents, the ED_{50} values varied from 0.06 to 0.10 mg/cm², and 0.15 to 0.20 mg/cm² for *An. moucheti* and *Mansonia* spp, respectively. Globally, the ED_{95} values of the three repellents were around 1mg/cm² except that of Cinq-sur-Cinq™ which was ≤ 0.3 mg/cm² in the case of *An. moucheti*. The estimated effective half-lifes of the three repellents were approximately between 3 and 5h. Our results highlight the heterogeneity in the response of different mosquito species when exposed to the tested insect repellents, showing the relevance of choosing and evaluating efficacy and persistence profiles of different formulations in specific environmental contexts.

576

A NOVEL DISSEMINATION TOOL FOR THE APPLICATION OF MOSQUITO LARVICIDES

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Uncertainty over the relative productivity of specific aquatic habitats, and the subsequent need to seek out, identify and treat all larval development sites makes the implementation of larviciding across large or spatially complex areas very challenging. A new technique that promises effective coverage of the most productive habitats may contribute greatly to the practicality and impact of such campaigns. Recent proof-of-principal studies show that natural behaviours of adult mosquitoes can be exploited for targeting larvicides to aquatic habitats. The treatment of mosquito resting places with pyriproxyfen; a persistent juvenile hormone analogue (JHA), results in the contamination of breeding sites as exposed resting adults subsequently disperse and oviposit. In the field, placement of JHA dissemination stations in 3 to 5% of the available resting area resulted in almost 100% coverage of aquatic habitats with JHA and overall reductions

in adult emergence of 42-98%. Pyriproxyfen has additional impacts on mosquito density through a reduction in the fecundity of exposed adults. Given the potential impact of this novel larviciding and chemosterilant technique, we report on the adaptation of a commercialised mosquito trap as a JHA dissemination tool. We describe the effective coverage of aquatic habitats achieved using these units in an urban environment in Peru, and their potential role within a push-pull strategy for Aedes control that is currently under development. In that strategy, mosquitoes are prevented from entering human habitations through the indoor residual treatments of repellent chemicals. A proportion of those repelled mosquitoes, and other individuals in the population, will enter strategically placed outdoor JHA dissemination units. Mosquitoes contaminated by those units will be sterilised and will also carry lethal doses of pyriproxyfen to their oviposition sites. Conventional "lure and kill" traps need to attract a large proportion of the mosquito population in order to be effective. The novel dissemination unit which we describe here is potentially far more efficient because the treatment of just a small proportion of the adult population can, through the persistence of the disseminated JHA, lead to an amplification in effective larvicide coverage.

577

LARVICIDAL AND ANTI-LA CROSSE VIRUS EFFECTS OF PLANT-DERIVED COMPOUNDS

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La Crosse virus (LACV) is an important cause of pediatric encephalitis in the Eastern United States. The mosquito *Ochlerotatus triseriatus* serves as the natural vector, while other species, including *Aedes albopictus*, are also competent vectors. Current larvicides have limited utility due to high cost and environmental and public health concerns. In these studies, we report larvicidal activity of plant-derived compounds that have previously been shown to have antiviral activity. The following compounds have been tested using *Ae. albopictus* as the test species: coffee extract, glycyrrhizic acid (from licorice), the flavonoids coumarin and morin hydrate (from multiple plants), and podophyllotoxin (from *Podophyllum peltatum*). Larvae were exposed to serial dilutions of each compound in triplicate, and mortality was compared to distilled water controls. Coffee extract and glycyrrhizic acid induced significant mortality in a dose-dependent manner. Coffee extract also had a direct virucidal effect on LACV. Future studies will be aimed at field tests to demonstrate effectiveness. These results indicate that plant-derived compounds may be used to control mosquitoes and may also be developed as antiviral treatments for LACV.

578

INSECTICIDE TREATED CAMOUFLAGE SCREENING REDUCES SAND FLY NUMBERS IN *LEISHMANIA*-ENDEMIC REGIONS IN KENYA

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Current U.S. military operations in deserts face persistent threats from sand flies that transmit human *Leishmania*. In this study we investigated the efficacy of artificial barriers treated with residual insecticide to potentially reduce the risk of human infection from leishmaniasis by reducing the number of sand fly vectors. Bifenthrin treated and un-treated camouflage netting was used to construct open-topped 10 x 10 feet enclosures 6 feet high at a field station in Marigat, Kenya where sand flies which readily transmit *Leishmania* are prevalent. Eight foot eucalyptus wood poles were used as the frame for the enclosures. Light traps baited with carbon dioxide were used as surrogates for human hosts and were operated overnight from 1530-0700 h on selected dates. We estimated sand fly mortality in 4 treated and 4 non-treated enclosures at various days post-treatment during hot-dry and hot-heavy rainfall conditions in Marigat, Kenya, by calculating the percent reduction in sand fly catch in treated enclosures as compared to untreated enclosures, a measure of relative efficacy of the treatment. We found a reduction in sand flies in treated enclosures when compared to untreated enclosures. Additionally, the difference in percentage found dead suggests that the toxic barrier is associated with a higher proportion of sand flies that die after being trapped, as compared to untreated enclosures. *Phlebotomus duboscqi*, *Phlebotomus martini*, and *Sergentomyia schwetzi* are thought to be the predominate sand fly species collected within the enclosures but identifications are pending. These results suggest that treated artificial barriers may be an effective tool to reduce *Leishmania* exposure to troops deployed in sand fly endemic regions, when used in conjunction with personal protective measures and other standard insect control measures.

579

HOW TO MAKE EVOLUTION-PROOF INSECTICIDES FOR MALARIA CONTROL

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Insecticides are one of the cheapest, most effective, and best proven methods of controlling malaria, but mosquitoes can rapidly evolve resistance. Such evolution, first seen in the 1950s in areas of widespread DDT use, is a major challenge because attempts to comprehensively control and even eliminate malaria rely heavily on indoor house spraying and insecticide-treated bed nets. Current strategies for dealing with resistance evolution are expensive and open ended, and their sustainability has yet to be demonstrated. Here we show that if insecticides targeted old mosquitoes, and ideally old malaria-infected mosquitoes, they could provide effective malaria control while only weakly selecting for resistance. This alone would greatly enhance the useful life span of an insecticide. However, such weak selection for resistance can easily be overwhelmed if resistance is associated with fitness costs. In that case, late-life-acting insecticides would never be undermined by mosquito evolution. We discuss a number of practical ways to achieve this, including different use of existing chemical insecticides, biopesticides, and novel chemistry. Done right, a one-off investment in a single insecticide would solve the problem of mosquito resistance forever.

580

GENETIC CONTROL OF *AEDES ALBOPICTUS* USING THE RIDL® SYSTEM

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Aedes albopictus is the secondary vector of dengue in the world after *Aedes aegypti*. It is also a natural vector of Chikungunya virus and a laboratory-competent vector of several other arboviruses. It is also a significant cause of biting nuisance where it has established. It is a highly invasive species, which has spread in the last 30 years from Southeast Asia and Pacific Islands to Europe, the Americas, Africa and the Middle East. Recently, *Ae albopictus* vectored a Chikungunya epidemic in the Pacific Ocean in 2006 and a Chikungunya outbreak in Italy in 2007. The spread of *Ae. albopictus* causes concerns regarding the possible emergence of dengue and Chikungunya in temperate countries. Current control measures have so far failed adequately to control *Ae. albopictus*; new control measures are much needed. The sterile insect technique is a proven method to control and potentially eliminate pest insect populations using irradiated sterile adults. However, the radiation-sterilisation is damaging

to some insects, including mosquitoes, making them less able to compete effectively for mates. RIDL® (Release of Insects carrying a Dominant Lethal) is an innovative approach to the control of mosquitoes and other insect pests that is an enhancement of the sterile insect technique. With this approach, sterility is induced by a dominant lethal gene inherited by the progeny, rather than irradiation. Here we report the development of a female-specific RIDL strain of *Ae. albopictus* with a tetracycline-repressible female flightless phenotype. Such a strain would allow easy and accurate separation of males from females and would be useful in a mass-rearing situation aimed at releasing males only. Homozygous eggs or pupae of this strain would be released into the wild; RIDL adult males would eclose ready to inseminate wild females and produce RIDL offspring which will die before adulthood, while RIDL females would be flightless and therefore incapable of biting or transmitting diseases.

581

BEHAVIORAL RESPONSE OF *CULEX QUINQUEFASCIATUS* TO DUET™ INSECTICIDE

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Duet™ insecticide contains the active ingredients prallethrin, sumithrin, and piperonyl butoxide. The excitatory effects of prallethrin reportedly cause resting mosquitoes to take flight and contact more droplets, thus improving insecticide efficacy. This premise was tested with female *Culex quinquefasciatus* using insecticide formulations that contained different combinations of these active ingredients. Ultra low volume (ULV) droplets were introduced into a wind tunnel. The response of individual resting mosquitoes was video recorded before, during, and after exposure, and behavior analyzed for excitation using behavioral analysis software. Mosquitoes exposed to insecticides moved faster when sprayed. Prallethrin produced increased flight activity during spray and sumithrin produced increased activity during the post-spray period. Mortality and the number and size of droplets on their bodies were also quantified, providing direct evidence between the volume of droplets and mortality.

582

PYRETHROID RESISTANCE AND THE COMPLEXITY OF TESTING INNOVATIVE PRODUCTS

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Long-lasting insecticidal nets were developed as a practical and sustainable solution to the problems encountered with conventional nets, which require regular re-treatment. The World Health Organization (WHO) guidelines stipulate that LNs must retain biological activity for at least 20 standard washes under laboratory conditions and 3 years of recommended use under field conditions. To date, pyrethroids are the only class of insecticide approved by the WHO for use on mosquito nets, for reasons of safety, efficacy, acceptability and cost. However, as concerns regarding the development of pyrethroid resistance in malaria vectors increase, the search for solutions to improve the efficacy of pyrethroids or find alternative insecticides has intensified. There are currently no guidelines for assessing the efficacy of nets designed for improved efficacy with pyrethroid-resistant vectors. Previous studies have assessed: a) mortality and/ or blood feeding rates of susceptible versus resistant strains in the laboratory and field b) bioefficacy of treated nets against characterised resistant strains in the laboratory, c) comparisons between experimental hut studies in areas of susceptibility with areas of resistance, d) mean mortality and blood feeding rates between treatments in an experimental hut study and e) *kdr* gene frequency of dead mosquitoes from experimental hut studies. The limitations of these studies in assessing whether a new insecticide or formulation can be considered for use against pyrethroid resistant malaria vectors will be discussed in the context of products that are available on the market for use today, and the consequences that may arise from incomplete or inappropriate evaluations of innovative products in the absence of appropriate guidelines.

583

DEVELOPMENT OF A BIOSENSOR CHIP FOR SNP GENOTYPING IN THE VOLTAGE GATED SODIUM CHANNEL GENE OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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The mosquito *Aedes aegypti* (Diptera: Culicidae) is the main vector of the four serotypes of dengue virus and each year the cases have been increasing, some of them are fatal. The resistance to pyrethroids insecticides used for control of this vector is increasing worldwide. One of the major mechanisms of resistance is the insensitive sodium channel, it prevents the rapid paralytic and lethal actions (knockdown) of all known pyrethroids, but does not diminish the efficacy of other insecticides. We have developed a Biosensor chip to simultaneously detect six point mutations in domain II of the voltage-gated sodium channel gene that potentially confer resistance to pyrethroids. These are Leu946Gln of segment 5, Ile1011Met, Ile1011Val, Val1016Gly and Val1016Ile of segment 6 and Phe1538Cys in the P-loop. This technique uses the ligase detection reaction (LDR) to interrogate single nucleotide polymorphisms (SNPs) in PCR products followed by hybridization and detection on a silicon wafer chip. Our technique allows for accurate and rapid genotyping of resistance SNPs in insecticide resistance management programs.

584

EVALUATION OF THE LARVICIDE PYRIPROXYFEN (SUMILARV 0,5 G) AGAINST *AEDES AEGYPTI* (DIPTERA: CULICIDAE) RESISTANCE TO TEMEPHOS AND DELTAMETHRIN

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Trujillo state, located in the Andean region of Venezuela, is considered to have a high incidence for dengue and populations of *Aedes aegypti* vector resistence to the temephos and delthametrin insecticide. The present work evaluated, in the laboratory conditions, the effect of an insect growth regulator, Sumilar 0,5G on the late 3ed/early 4th *A. aegypti* larvae (strains PTO and CUA resistences to temephos and deltamethrin respectively). The insecticide susceptible reference strain Kockefeller was used. In the both isolated, the mortality in pupe phase was significantly larger that in the larvae phase. The Inhibition of the emergency in the PTO was of 0,69 for the concentration of 0,01 ppm and 0,77 with the concentration 0,05 ppm while for the strain CUA the inhibition of the emergency for both concentrations was of 1. These results have to be considered at the moment design vector control programs and insect growth regulator. Sumilarv 0,5G could be a good alternative for controlling *A. aegypti* pupae.

585

DETERMINANTS OF FOCAL INSECTICIDE RESISTANCE OF *AEDES AEGYPTI* IN THE PHILIPPINES

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This project addresses the relationship between insecticide use and the level of insecticide resistance in the main dengue (DEN) transmitting mosquito, *Aedes aegypti*, in the Philippines. Dengue fever (DF) and dengue hemorrhagic fever (DHF) are a major health burden, especially in developing countries. DEN virus is spread to people through the bite of an infected mosquito, primarily *Ae. aegypti*. In many countries entomologists have observed variation in levels of resistance to individual insecticides in different parts of the same municipality, including recently in Manila, Philippines. While focal insecticide resistance patterns are well established, there have been no systematic studies of human insecticide use behavior and its influence on resistance. *Ae. aegypti* eggs were collected from four dengue endemic areas of the Philippines, reared to adult, and bioassayed to determine resistance to 4 insecticides. Two sites were in densely populated portions of Metro Manila with high dengue incidence and two were in peri-urban communities of central Luzon with moderate dengue transmission. The CDC Bottle Bioassay was used to measure insecticide tolerance to two pyrethroids (permethrin and deltamethrin), an organophosphate (temephos), and an organochloride (DDT). Public health, household, and industrial insecticide use were documented at each site. A survey, household insecticide census, in-depth interviews, community mapping, direct observation, and other rapid ethnographic methods were conducted to quantify insecticide use and determine the manner and purpose of insecticide use. Participants included members of the study neighborhoods, health offices, local governments, civic organizations, schools, and businesses. Public health and household level insecticide use were evaluated and correlated with the specific resistance patterns observed at each site to determine which behaviors most impact insecticide resistance. Recommendations for judicious use of insecticides to prevent insecticide resistance are proposed.

586

OVIPOSITION SITE SELECTION IN THE DENGUE VECTOR, *AEDES AEGYPTI*

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We tested the hypothesis that ovipositing *Aedes aegypti* actively select containers that are most suitable for development of their progeny. We surveyed 389 containers in 277 households in Iquitos, Peru, recording the number of *Ae. aegypti* eggs laid per day over a 3-day period and container characteristics such as size, material, location, water management, organic material, solar exposure, abundance of conspecific larvae, and presence of *Culex spp.* larvae. Odds of females laying eggs in containers increased with presence of conspecific larvae and *Culex* larvae. Average daily number of eggs laid was positively correlated with abundance of conspecific larvae, presence of *Culex* larvae, container circumference, and unmanaged water. Suitability of 73 containers for larval development was assessed by conducting starvation assays with 3rd instar larvae removed from those containers. We found no association between number of eggs laid in a container and length of time larvae from that container survived without food ($R^2 < 0.01$, P-value = 0.69). To assess whether oviposition choice is associated with improved larval survival and/or increased offspring size, four container types were set out in each of 20 houses: large and small containers, each with managed and unmanaged water. We introduced cohorts of 25 1st instar larvae from paired laboratory matings into each container and monitored oviposition daily for 23-26 days. Using microsatellites to identify introduced individuals, we will compare survival rates to pupae and emerging adult size. Improved understanding of *Ae. aegypti* oviposition behavior will enable public health officials to better predict how populations will respond to dengue control measures that target specific oviposition sites for treatment or removal. If oviposition decisions are made to increase offspring fitness, females are likely to adapt to a changing landscape of available containers, such that removal of the most productive containers will not lead to a simple, proportional reduction in mosquito population size.

587

IMPACT OF LARVAL MOSQUITO COMPETITION ON COMPONENTS OF VECTORIAL CAPACITY

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Much is already known about the influence of ecological factors on adult mosquito morphology and physiology as well as the genetic barriers to pathogen replication within adult mosquitoes. However, we have a poor general understanding of how biotic interactions among larvae, specifically inter- or intra-specific competition, impact innate barriers to pathogen development and transmission. The focus of this study is to evaluate the impact of a highly competitive environment for larvae on components of vectorial capacity for adult *Aedes aegypti* and *Ae. albopictus*, species that are known competitors as well as disease vectors. Five replicates of a larval competition experiment using *Ae. aegypti* and *Ae. albopictus* from Florida were run. Six larval density-species combinations, in 400ml cups with deionized water and live oak leaves (Florida), were (ALB:AEG): 40:0, 20:0, 20:20, 10:10, 0:20, 0:40. As adults eclosed, they were placed into individual cages, provided with continuous access to sugar, and kept at 26°C, 90%RH with a 14:10 L:D cycle. We determined treatment effects on adult longevity and frequency of blood feeding. Females were given an opportunity to blood feed on a mouse for 15 minutes every 4 days. Daily survival rates, feeding success, and numbers of eggs laid were recorded. At death, females were dried and wings measured. There were significant effects of competitive treatment on percent survival of larvae to adults for both *Ae. aegypti* ($F_{3,12}$=9.92 P=0.0014) and *Ae. albopictus* ($F_{3,12}$=6.96, P=0.0057). High inter- and intraspecific densities reduced adult longevity of *Ae. albopictus* and *Ae. aegypti* by 26% and 57%, respectively, suggesting important effects of competition on probability of daily survival, an important component of vectorial capacity. Our results contribute to a growing body of research on how the ecological effects on larvae affect components of vectorial capacity of adult mosquito populations.

588

LONGEVITY OF *ANOPHELES GAMBIAE* S.L. UNDER NATURAL CONDITIONS USING A MODIFIED MARK RELEASE RECAPTURE APPROACH

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Malaria is a major public health problem in Africa. Important determinants of malaria transmission are the same traits that constitute mosquito fitness, e.g., longevity and life-long fecundity. Despite efforts to obtain robust estimates of the distributions of these parameters under natural conditions, available estimates provide the average values at best, but their distributions, which have greater epidemiological implications remain unknown. To obtain this information, we conducted an unusual mark release recapture experiment (MRR) in a small isolated village in Mali. Each mosquito was marked with a unique code so it can be released indefinitely and the experiment was conducted over two months, capturing mosquito

every other day in all houses and releasing each one in the same point it was captured. Using emergence traps, we target adults as they emerge from larval sites so their age is known throughout. These features increase recapture rates and allow obtaining a realistic longevity distribution in relation to age. We captured marked and released 2397 males and 4534 females - each. Almost 30% of the mosquitoes were collected as they emerged from larval sites, so their age was known throughout the experiment. We recaptured 248 mosquitoes, 58 males and 190 females (some of which were recaptured twice, and a couple - three times). Survival estimates derived based on MARK revealed that the probability to capture surviving mosquitoes was low (0.03 and 0.01 for females and males, respectively), suggesting large total population size. Overall, daily survival of males (79%) and females (83%) was similar. Extensive use of ITNs in this village (>90% of houses had one or more) may have reduced survival. During the rainy season survival was higher than in the early dry season, partly explaining the decline in population size. Notably, survival after the first eight days was higher than survival of younger mosquitoes.

589

SPATIAL CLUSTERING OF WEST NILE VIRUS INFECTION IS ASSOCIATED WITH COMBINED SEWER OVERFLOW CREEKS IN URBAN ATLANTA, GEORGIA

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The distribution of West Nile virus (WNV) infection in humans is generally clustered in space and time, with highest concentrations of cases occurring in urban areas. At present, the factors that favor transmission and amplification of WNV and the heterogeneous pattern of infection within urban environments are poorly understood. The objective of the present study was to describe and quantify the spatial distribution of WNV infection in humans, birds and mosquitoes for the period 2001-2007 in Fulton County, the most populated district of metropolitan Atlanta, and the county with the highest numbers of WNV in Georgia. Local spatial statistics (Morans's I tests) were applied to human, bird and mosquito surveillance data for the period 2001-2007; a Geographically Weighted Regression was used to determine the spatial association between infection clustering and selected environmental and demographic factors. WNV infection was found to be spatially clustered in close proximity of Combined Sewer Overflow creeks, where organically rich sewer discharges (highly suitable for Culex quinquefasciatus mosquito breeding) coupled with the presence of forested and residential/recreational areas resulted in near-optimal conditions for virus transmission. In the US, some 40 million people in 772 cities from 32 states live in cities with combined sewer systems. If the association between sewage systems and WNV holds in other cities, vector control and environmental management can be targeted accordingly to reduce virus amplification and transmission.

590

BIOACOUSTICS AND COURTSHIP IN *AEDES AEGYPTI* AND *ANOPHELES GAMBIAE*

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Currently, we do not understand key elements of mosquito fitness, despite the importance of fitness for successful SIT and transgenic control strategies. Female flight tone has long been known to be important for localization and species identification in male mosquitoes. In earlier work, we demonstrated that opposite-sex pairs of *Aedes aegypti* converge at harmonic frequencies and that mated females were less likely to respond to male stimuli. These experiments led us to predict that harmonic convergence played a role in mosquito pre-copulatory behavior. Here we present evidence from two medically important species, *Ae. aegypti* and *An. gambiae,* that acoustic signals contain fitness status information that is perceived by conspecifics. Using playbacks, we measured the response of male and female mosquitoes to large and small potential mates and demonstrated that males change the nature of their response depending on the perceived size of a female. Next we tethered females to a strand of hair and recorded acoustic interactions with free flying males. Our results suggest that harmonic convergence determines the outcome of mating attempts. Finally, we will present results on the direct and indirect benefits received by females and their relationship with parameters of male signaling.

591

INNATE HOST SELECTION BY *CULEX PIPIENS* SAY (DIPTERA: CULICIDAE)

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Contact rates between disease vectors and hosts are critical drivers of vector-borne disease transmission. Heterogeneity in contact rates may result in certain hosts generating the majority of infections, a phenomenon known as 'super-spreading'. Contact rates are influenced by environment, host availability, and innate vector host-preferences. Traditional means for determining mosquito host-preferences are to estimate the proportion of blood meals on certain hosts or to calculate a feeding index, which accounts for host abundance. In the case of West Nile virus (WNV), field studies suggest that *Culex pipiens* feed on American robins (*Turdus migratorius*) more often than expected based on their abundance. Although the feeding index can be defined as a 'preference', the vector's innate behavioral response remains confounded by environmental factors. The goal of our study was to determine the level of innate preference that Cx. pipiens exhibit for robins when environmental factors were removed. We conducted a series of host choice experiments, in which *Cx. pipiens* were introduced into a large mosquito-proof outdoor enclosure containing two bird-baited mosquito traps. We measured 'activation' as the proportion of mosquitoes which entered either trap, and 'preference' as the probability that mosquitoes entered a robin-baited trap when paired with either a European starling (*Sternus vulgaris*), a house sparrow (*Passer domesticus*) or another robin (control). Overall mosquito activation was 10.54 ± 0.01%, and not different between pairings ($p>0.05$). We found that *Cx. pipiens* displayed significant preference for robins over starlings (3:1) and house sparrows (2:1). No difference was detected when mosquitoes chose between two robins. We did not find any effect of the birds' weight, age, sex, date, start-time, temperature, humidity, wind-speed and mosquito age on the probability of choosing a robin. We demonstrated that *Cx. pipiens* are activated by host odors and exhibit significant innate preferences for certain host species. The tendency to select robins in these experiments is lower than observed field feeding indices, suggesting that while this response is modulated by the environment, a strong innate effect remains. Separating innate from environmentally-driven components of host preference are essential for predicting mosquito behavior in new environments or making predictions regarding risk of vector-borne disease.

592

ASSESSING RISK IN FOCAL ARBOVIRAL INFECTIONS: ARE WE MISSING THE BIG OR LITTLE PICTURE?

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Focal arboviral infections affecting a sub-set of the overall population present an often over-looked set of challenges in the assessment and reporting of risk and the detection of spatial patterns. Our objective was to assess the possible variation of risk when using different at-risk populations and geographic scales for the calculation of incidence risk and the detection of disease clusters. We explored these variations using a pediatric arbovirus, La Crosse virus, as our model. Descriptive and cluster analyses were performed on probable and confirmed cases of La Crosse virus infections reported to the Tennessee Department of Health from 1997-2006, using three different at-risk populations and two geographic levels to assess the variation in incidence risk and to investigate evidence of clustering using both global and local spatial statistics. We determined that the most appropriate at risk population to calculate incidence risk, and to assess evidence of clustering was 0-15 year old population cohort. Based on our findings, the most appropriate geographical level to conduct spatial analyses and report incidence risk was the census tract level. Our results indicate the possibility of missing disease clusters resulting from performing incidence risk investigations of focal diseases using inappropriate at-risk populations and/or large geographic scales. Public Health efforts to improve both disease surveillance and health planning would be better improved through the assessment of risk in well defined at-risk populations and geographic scales. This ensures that public health efforts to control disease occurrence are as efficient as possible.

593

EFFECTS OF DIFFERENTIAL ITN COVERAGE ON MOSQUITO ABUNDANCE IN FOUR ECOLOGICAL SETTINGS IN COASTAL KENYA

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Recent rapid scaling up of Insecticide treated nets (ITN) coverage in Kenya has resulted in coverage of >60%. High ITN coverage has been shown to reduce dramatically malaria transmission in several parts of sub-Saharan Africa, as demonstrated in a number of randomized trials. Intense ITN use results in decreased numbers not only of mosquitoes, but also of parasites both in humans and in mosquitoes. As part of a polyparasitism study in coastal Kenya, we determined the effects of heterogeneous ITN coverage on mosquito abundance and sporozoite rates in four sites: estuary, coastal plain, coastal slope, and inland semi-arid ecological areas. A longitudinal survey was conducted in eight villages (two in each of the four ecological settings) in the south coast of Kenya. Adult mosquitoes were collected indoors using pyrethrum spray collection and outdoors using clay pots located adjacent to houses. *Plasmodium* species-specific malaria sporozoite rates were determined using ELISA. ITN coverage was determined by administering a structured questionnaire to a randomly selected sample of heads of households in each of the 8 villages. *Culex* spp. were the principal species collected, followed by low densities of *Anopheles gambiae* s.l. and *A. funestus*. The malaria vector mosquito population densities remained low and variable throughout the study period in all four ecological settings. Clay pot collections correlated well with PSC although they tended to underestimate mosquito densities when pyrethrum captures were large. Rainfall pattern corresponded with peaks of mosquito population abundance. An inverse relationship was observed between entomological inoculation rates and ITN coverage. Achieving high and even ITN coverage, combined with proper adherence could contribute to the prevailing downward trend in malaria prevalence.

594

COMBINING ENVIRONMENTAL MANAGEMENT WITH INSECTICIDE-TREATED NETS FOR MALARIA CONTROL

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Integrated vector management (IVM) for malaria control has received a lot of recent interest for two main reasons: first, it is hoped that using multiple techniques in combination will act synergistically to enhance disease control efforts and, second, it will reduce the dependence of current control measures on insecticides. Given WHO's current target of 80% human coverage with insecticide-treated nets (ITNs), it would be logical to assess which alternate measures would enhance malaria control and prevention programs that already use ITNs most effectively. We present a simple, biologically-driven mathematical model of malaria transmission to investigate the utility of combining mosquito larval resource reduction with ITNs. We select this type of environmental management to compliment ITNs because of a potential secondary mode of action that both control strategies share. In addition to increasing vector mortality and reducing human-vector contact rates, ITNs reduce the rate at which female mosquitoes locate human hosts for blood feeding, thereby extending their gonotrophic cycle. Similarly, while reducing adult vector emergence and abundance, source reduction of larval habitats may prolong the gonotrophic cycle by extending delays in locating oviposition sites. Both control strategies therefore have the potential of reducing the bite rate by limiting the availability of mosquito resources. We illustrate, however, that source reduction of larval habitats only operates through this secondary mode of action below a critical threshold habitat density. Hence, we show how this strategy becomes effective only when larval habitats are scarce. When used in combination with ITNs, larval habitat resource management generally yields modest additional benefits. This is, however, dependent on the environmental settings with regards to the density of both humans and larval habitats. We describe the necessity of tailoring both vector management strategies to specific environmental settings in order to optimize control.

595

IMMUNOLOGICAL PRIMING IN *ANOPHELES GAMBIAE*: CAN MOSQUITOES 'LEARN' FROM A CHALLENGE WITH *PLASMODIUM*?

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The insect immune system is thought to be completely innate and incapable of mounting an adaptive immune response. But recent studies have shown that priming of *Drosophila melanogaster* with a sub-lethal dose *of S. pneumoniae* protects against a lethal second challenge of *S. pneumoniae.* We explored the capacity of adult female *Plasmodium*-infected *An. gambiae* mosquitoes to combat a re-challenge with *Plasmodium*, one or two weeks after the first infection. Two groups of mosquitoes were fed on the same *Plasmodium berghei*-infected mouse. One group (Challenged) was kept at a permissive temperature (21°C) that allowed *P. berghei* ookinete development and midgut invasion. The second group (Naïve) was placed at a non-permissive (28°C) temperature immediately after feeding on the infected mouse. When re-infected with *P. berghei,* challenged mosquitoes displayed a significantly reduced infection compared to the naïve group. This enhanced immunity persisted for almost the entire life span of the mosquito. A similar phenomenon was also observed in mosquitoes challenged with *Plasmodium falciparum*, the human malaria parasite. Administration of antibiotics to both the

groups of mosquitoes, before the first or second challenge abolished this enhanced immunity to *Plasmodium*. This effect, therefore, was found to be dependent on the presence of endogenous bacterial gut flora, which had to be present at the time of the first infection to establish the response as well as at the time of the second infection to elicit this immune response. Our findings indicate that adult female mosquitoes challenged with *Plasmodium* are able to 'learn' and respond more efficiently to subsequent challenges. This effect on *Plasmodium* is indirect and mediated by a priming response to bacteria. Several immune markers are being screened for transcriptional induction in mosquitoes from the two groups. Studies are underway to establish the mechanism, which determines such a 'learned' response in challenged mosquitoes.

596

MOSQUITOES AND DENGUE VIRUSES IN SCHOOLS IN MÉRIDA, MEXICO

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As a follow-up to a previous study demonstrating that dengue virus (DENV)-infected *Aedes aegypti* are common in the homes of dengue patients in Mérida, Mexico, we conducted a similar study focusing on risk for exposure to DENV-infected *Ae. aegypti* in schools. Mosquitoes were collected by backpack aspiration from October-December 2008 in 24 schools, ranging from kindergartens to colleges, in the southern part of Mérida. These efforts yielded a total of 3,803 mosquitoes including 3,032 *Culex quinquefasciatus* (79.7% of total mosquitoes), 567 *Ae. aegypti* (14.9%), 203 *Ochlerotatus taeniorhynchus* (5.3%), and 1 *Oc. trivittatus* (0.1%). Of the 261 *Ae. aegypti* females captured, most were collected from classrooms (53.6%) followed by bathrooms (16.9%), storage rooms (12.6%), offices (12.3%), and other room types (4.6%). This demonstrates that students, teachers, and administrative personnel all are at risk for exposure to *Ae. aegypti* bites in the schools. Seventy-five pools of *Ae. aegypti* females were examined for presence of DENV RNA by RT-PCR. Nine of these pools contained DENV-1 RNA and one pool contained with DEN-4 RNA. Mosquito pools containing DENV RNA were recovered from all five room types mentioned above. Although the prevalence of mosquito pools containing DENV RNA in the schools (13%) was similar to that observed previously for homes of dengue patients from September-December 2007 (10%), the number of infected mosquitoes per person, and hence the risk of an individual being bitten by an infected female, is far lower in the schools because of the greater number of persons frequenting a school compared to a home. On the other hand, schools may serve as nodes for transmission leading to DENV being spread widely within the school district. Our findings underscore the need for further studies to more clearly determine the relative importance of homes versus schools, and the relative roles of small children, school-age children and adults, in DENV transmission dynamics in Latin America.

597

MONITOR AND OPTIMIZE DURABLY THE USAGE OF INSECTICIDE TREATED NETS WITHIN HOUSEHOLDS WITH DETECTOR FOR REDUCING MALARIA TRANSMISSION, IN CENTRAL IVORY COAST: RESULTS OF PRELIMINARY TRIALS

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Mosquito the control measures practiced currently have lead to the development of insecticide resistant mosquito populations. With regard to malaria, sleeping under long lasting insecticide treated net (LLINs) treated net has shown a track record of reducing malaria-related morbidity. However, in some regions, when nets are not properly used; significant reductions are not obtained concerning malaria transmission. Against this background, we propose to validate the use of an entrained electronic motion detector, ton on-invasively monitor the usage and householder washing patterns of LLINs in a malaria endemic setting, and then use this system to estimate the impact of mosquito density and temperature on LLIN usage as proof of concept for the technology. Detectors have been attached to nets which have been offered to volunteers who accepted to sleep under nets from October to December 2008. Detector record data on 3 axes (x, y, z) concerning the usage of nets over an extended periods. The values on these data are closely linked and dependent of motions realized by the users. This initial laboratory work has established the optimal location of the loggers on the nets to monitor, on a daily basis, the time, speed and format in which nets were placed over the beds and the time at which the user entered and exited the net. For example, tying the net after use for storage during the day is sensed by the loggers placed approximately half way down the long side of the net as a relatively long motion (6 to 20 seconds) and the differences between means recorded every 0.1 s are highly significant. In contrary, pulling down a net lasts 0.5 to 3s, and the differences between means recorded every 0.1 s are low. Data recorded shown also that figure for net occupied is completely different from the one of unoccupied net. Meanwhile, we do not anticipate that the visibility of the loggers will directly affect bed net usage pattern. Attachment of the loggers onto the net will be in such a format to facilitate their easy removal by the research team without taking the net away.

598

MALARIA TRANSMISSION ALONG THE NIGER RIVER IN A SUDAN SAVANNA AREA OF MALI

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In Sudan savanna areas of Mali, malaria transmission is seasonal. However, we previously reported productive larval habitats along the river Niger, which was responsible for abundant vector population in fishermen's hamlets during the dry season. The aim of the current study was to determine how this situation impacts the prevalence of malaria infection in children <5 years old. Entomological and parasitological cross-sectional surveys were carried out in two hamlets (Bozokin and Fourda) each less than 1 km from the river, and the village of Kenieroba at 2-3 km of the river. Entomologic surveys were conducted in March-April (dry season), June (beginning of rainy season) and October (end of rainy season) over two years. Parasitological surveys were carried out in April, June and October. *An. arabiensis* (7%) and *An. gambiae s.s.* (93%) were the major malaria vector species. Mosquito density remained relatively stable in the two hamlets with highest densities observed in March and October (30-40 bites/person/month). The density was markedly rainfall dependent in Kenieroba with a peak in October (50-60 bites/person/month) and lower during the dry season (5 -10 bites). The prevalence of *P. falciparum* infection was similar in the three villages at the end of the rainy season (40-45%), but lower than the expected prevalence in typical areas savanna areas (60-70%). During the dry season, the prevalence of *P. falciparum* infection decreased sharply in Kenieroba (19%) while it remained relatively high in Bozokin (50%) and Fourda (30%). The highest prevalence of gametocyte carriage in children (30%) was observed during the dry season in Bozokin and correlates with high density in vector population. These results suggest that study villages may contribute to spread both mosquitoes and malaria infection during the subsequent rainy season in remote communities. As a large number of people live around the main rivers, vector control strategies should pay special attention to riverbank malaria transmission.

599

SPATIAL VARIATION OF HOST SELECTION IN *CULEX PIPIENS* MOSQUITOES

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Recent field studies are measuring feeding patterns of mosquitoes using advances in molecular blood meal identification and quantify host selection based on measures of availability. These studies incriminate vectors responsible for arbovirus transmission and implicate species responsible for host selection. However, most of these studies aggregate data across samples collected from different geographic areas due to limited sample sizes. We performed a mosquito blood meal analysis integrating host-feeding patterns of *Culex pipiens* (n = 750) with measures of host availability from 10 different sites in a West Nile virus-endemic area of suburban Chicago, Illinois, during 2005-2008. We will present both the spatial variation in host selection and the amplification fraction of the avian community at each site. Results demonstrate the importance of considering spatial variation when measuring feeding patterns and host selection, especially when genetic substructuring in the Cx. pipiens complex is known to influence host preference.

600

BEHAVIORAL CHANGES OF FEMALE *AEDES AEGYPTI* IN RESPONSE TO VARIOUS COMBINATIONS OF MATERIAL TEXTURES, COVERAGE, AND DOSES OF INSECTICIDES USING A NOVEL LABORATORY ASSAY

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Current focus of dengue prevention is targeted at the vector population. Adult control focuses mainly on the toxic action of chemicals. However, research shows that killing insects may not be necessary for effective vector control. Other approaches to reduce man-vector contact at the house level exist and might be sufficient. This includes initiating a repellent effect, preventing house entry; and/or irritant effect, causing an escape response prior to mosquitoes biting humans indoors. Such an approach is currently being evaluated for a push-pull control strategy for *Aedes aegypti* by targeting preferred house entry portals and indoor resting sites to make them unsuitable. The goal of the larger program is to drive the development of innovative control strategies using minimal chemical dose for cost-effective approaches in reducing indoor densities of vector populations.This study reports on the behavioral changes of two geographically distinct (Thailand and Peru) female *Ae. aegypti* strains in response to varying material textures, colors and coverage, treated with standard vector control compounds at different doses. Baseline data were generated using chemical-free material to quantify trends in resting patterns, escape (i.e., irritancy) and entry (i.e., repellency) rates using a novel laboratory "box" assay. Changes in mosquito behavioral patterns were then evaluated upon exposure to focal treatment of preferred resting sites and/or assay entry portals. Knockdown and 24 h mortality rates were evaluated under each treatment condition. Results of this study are being validated under field conditions in both Thailand and Peru using an experimental hut study design and will ultimately guide the optimal configuration of treated material for the push-pull strategy.

601

IMMUNOGENICITY OF *ANOPHELES ALBIMANUS* SALIVA IN MALARIA ENDEMIC AND NON-ENDEMIC AREAS

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In the process of mosquito feeding, saliva helps in the uptake and digestion of the blood-meal. Previously reported studies have shown that mosquitoes inject pharmacologically active compounds contained in their saliva into the host. These salivary components include several proteins that have been shown to elicit strong immune responses. The presence and level of human anti-saliva antibodies are highly dependent on the mosquito population, representing a measure of the level of mosquito bite exposure in humans. One of the principal malaria vectors in South America and the Caribbean is *Anopheles albimanus*. The purposes of this study were: 1) to evaluate the level of IgG and IgM antibodies against *An. albimanus* salivary gland; and extract as a measure of individual mosquito bite exposure and malaria risk in areas endemic for malaria and 2) to determinate if individuals living in places were *An. albimanus* is not endemic presented antibodies that react against saliva from this mosquito. A total of 113 individuals were included in the study: 21 from Haiti, 30 from Guinea, 30 from the United States, and 32 from Colombia.

602

ALGINATE-ENCAPSULATED FORMULATION OF BACTERIA THAT ATTRACT GRAVID *AEDES AEGYPTI* AND *AEDES ALBOPICTUS*

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In laboratory behavioral assays, infusions made from senescent leaves of bamboo (*Arundinaria gigantea*) are highly attractive to gravid *Aedes (= Stegomyia)* mosquitoes. A mix of 14 bacterial species isolated from bamboo leaf infusion, and cultured in R2A media attracted significant numbers of gravid females. However, when single species isolates were bioassayed, some bacterial species were found to be highly attractive while other species repelled female mosquitoes. Sodium alginate was used to formulate a mix of the five most attractive bacteria. Freshly prepared bacterial beads were highly attractive in behavioral assays. An alginate bacterial bead formulation with an extended shelf-life was produced using a freeze-drying procedure. Once hydrated, the bacterial beads were again highly attractive to gravid mosquitoes. The effects of incorporation of solid support materials, such as powdered milk and microcrystalline cellulose, on the viability of bacteria in freeze-dried formulations will be presented. Traps are used by abatement programs for surveillance and control of dengue virus vectors, and alginate bacterial beads are a promising formulation that can increase the numbers of gravid *Aedes* females that are attracted to traps.

603

A COMPARATIVE CLINICAL STUDY OF INVASIVE PNEUMOCOCCAL DISEASE CAUSED BY PENICILLIN-RESISTANT AND PENICILLIN-SENSITIVE *STREPTOCOCCUS PNEUMONIAE* IN THAILAND

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This study focused on determining the clinical difference between invasive pneumococcal disease (IPD) caused by penicillin-resistant and penicillin-sensitive *Streptococcus pneumoniae*. Patients with invasive pneumococcal diseases seen during January 1996-December 2007 at 3 hospitals were studied. Comparison of clinical epidemiology between those with penicillin-resistant *Streptococcus pneumoniae* (PRSP) and penicillin-sensitive *Streptococcus pneumoniae* (PSSP) strain were done. There were 69 patients with IPD, 25 were infected with PRSP and 44 with PSSP strains during the period of study**.** Sex, mean age, underlying diseases and seasonal variation did not statistically differ between the two groups. The clinical efficacy determined by duration until defervescence, duration of hospitalization and clinical outcome did not have statistical difference in both groups. Minimum inhibitory concentration (MIC) of antibiotics of pneumococcal isolates were examined. PRSP isolates had resistance to cephalosporins and meropenem 15.8% and 38.9% respectively, but all of them were sensitive to vancomycin. The predominant serogroups in PRSP isolates were 23 and 19 while in the PSSP group were serogroups 6 and 14. In conclusion, there were no significant differences in clinical epidemiology of IPD in both PRSP and PSSP group.

604

IDENTIFICATION AND COMPARISON OF *MYCOBACTERIUM LEPRAE* GENOTYPES IN TWO DIFFERENT GEOGRAPHICAL REGIONS OF COLOMBIA

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Patients from Agua de Dios, Barranquilla and Cartagena cities and neighboring towns were enrolled during 2006-2007 for molecular epidemiology studies. Slit skin smears or biopsies were obtained from newly detected untreated patients, and those undergoing multidrug therapy. The samples (n=38) were processed to develop molecular procedures including strain typing based on *M. leprae* variable number of tandem repeat (VNTR) analysis and single nucleotide polymorphisms. Differences or similarities between strain types from the North East (n=20) and Central regions of Colombia (n=18) were examined. The alleles at two loci, 27-5 and 12-5 were different in the *M. leprae* in the two regions. Furthermore, there was strong association of the 2 locus alleles with the SNP types. The 4-5 combination of alleles was associated with the SNP type 3, while the 5-4 combination was mostly associated with SNP1, 2 or 4. The SNP type 4 *M. leprae* isolates were seen in patients in the North East, but not in the central part. The other microsatellite loci are more useful for further intra-population differentiation.

605

TUBERCULOSIS RELATED STIGMA EPIDEMIOLOGY AND RISK FACTORS

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Stigma is a barrier to tuberculosis (TB) control but is poorly characterized in the settings where most TB occurs. We therefore investigated the prevalence and nature of TB-related stigma and characterized risk factors for stigmatization in communities with high TB prevalence. This nested cohort study involved 2,253 residents in TB-affected households: TB patients (n=787) and their healthy household contacts (n=1,466) in 16 adjacent peri-urban shantytowns in Lima/Callao, Peru. Participants were interviewed in the final month of TB treatment. Nurses interviewed each participant and administered a questionnaire with 22 questions concerning perceptions and experiences of TB-related stigma in three settings: home, community and work. 71.6% (563) of patients and 75.2% (1,694) of their contacts reported experiencing TB-related stigma. TB patients felt significantly more stigmatized than their contacts ($p<0.001$), and stigma was 15.2 times more likely to be reported by contacts of patients who also experienced stigma (OR=15.2; CI=10.7-21.6; $p<0.001$). Women were more stigmatized at home ($p<0.001$) and in the community ($p<0.01$), whereas men were more stigmatized at work ($p=0.01$). Women were more likely to report mistreatment at home (OR=2; CI=1.4-2.9; $p<0.001$), avoidance by the community (OR=2.1; CI=1.1-4.3; $p=0.04$), and mistreatment by the community (OR=3.1; CI=1.3-7.7; $p=0.01$). Men were more likely to report that TB-stigma caused a change in responsibility at work (OR=1.7; CI=1.1-2.5; $p=0.02$). Lower income and fewer years of education were associated with stigmatization in general and particularly avoidance at home and by the community, having lost work, responsibility or a change in function at work (all $p<0.05$). In conclusion, experience of TB-related stigma was frequent in patients and people living with them and was associated with low-income, limited-education, and gender-influenced daily environments (home, community and work). Stigma is a social barrier that hampers TB control and interventions to address stigmatization should focus on these high-risk groups.

606

THE IMPLICATIONS OF 17,000 FIRST-LINE AND 11,800 SECOND-LINE DRUG SUSCEPTIBILITY TESTS FOR SELECTING OPTIMAL TB TREATMENT

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The empirical patterns of treatment for TB proposed by the World Health Organization (WHO) should be reviewed in setting with frequent drug resistant TB such as Peru. Rapid drug-susceptibility testing to isoniazid (INH) and rifampicin (RIF) is recommended, but the implications of the results have not yet been defined. We reviewed susceptibility tests performed at the National Institute of Health in Peru: 17,022 first line and 11,816 second line drugs sensitivity tests (DST) from 2005 to 2008. Drug-susceptibility results were classified into 4 possible phenotypes: (1) sensitive to INH and RIF, (2) Resistant to INH and sensitive to RIF, (3) Sensitive to INH and resistant to RIF and (4) Resistant to INH and RIF (MDR). For each resistance phenotype, first and second line drug susceptibilities were determined. These data were used to define optimal

empiric treatment regimes based on the rapidly available INH and RIF susceptibility tests. RESULTS: Of the 17,022 tests, 54.5%, 10.2%, 2.50% and 32.8% had susceptibility phenotypes 1,2,3 and 4, respectively. The proportion (%) of susceptibility to anti-tuberculous drugs for phenotypes 1,2,3 and 4 were, respectively: Ethambutol (EMB) 99.4, 91.8, 90.5 and 58.6; Pirazinamide (PZ) 99.7, 90.8, 85.0 and 59.3; EMB & PZ 99.1, 83.8, 78.9 and 37.9; EMB or PZ 100, 98.3; 96 and 78.3; Streptomicyne 87.8, 44.7, 78 and 33.9; Ciprofloxacine 99.7, 98.5, 98 and 92.0; Kanamicine 99.6, 96.4, 95.3 and 80; Capreomicine 99.8, 98.2, 97.5 and 86; Ethionamide 97.8; 77.2; 81.3 and 72.5; PAS 99.9; 95.1; 99.3 and 94.7; and Cicloserine 99.9, 99.5, 99.7 and 99.1. When INH and RIF were susceptible, 99% of the strains were also susceptible to PZ and EMB. However, when resistance to isoniazid is detected, it should be replaced by a quinolone with RIF, PZ and EMB. If there is RIF resistance, then this drug should be replaced by a quinolone and capreomycin. For MDRTB phenotype, a quinolone should be used plus capreomycin, PAS, cycloserine, EMB and PZ. Streptomycin and ethionamide had the highest resistance in Peru and they should not be used in the initial schemes. The results of this study suggest that a new model should be adapted in Peru to replace empirical primary schemes identified by WHO. This should be based upon rapid early INH and RIF susceptibility testing. Patients with TB resistance to INH or RIF should receive modified therapy, supported by extended drug susceptibility testing to allow individualized therapy.

607

THE EPIDEMIOLOGY OF HUMAN LEPTOSPIROSIS IN TRINIDAD AND TOBAGO BETWEEN 1996-2007: A RETROSPECTIVE STUDY

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A retrospective study to describe the epidemiology of leptospirosis in Trinidad and Tobago during 1996-2007 was carried conducted. All confirmed cases of leptospirosis were analysed according to seasonality, sex, age and geographic distribution. A total of 278 cases was recorded, with an average annual incidence rate at 1.84 per 100 000 population. 75% of cases occurred during the wet season, with the highest number of cases recorded in November. A positive correlation was found between number of cases and rainfall amount. Males made up 80% of all cases, and the overall male:female ratio was 4.6. The total case fatality rate was 5.8%, with deaths among males four times more common than in females. Infection was greatest in the 10-19 age group and lowest in the 0-9 age group. The total prevalence was 22 per 100 000 population, with the highest prevalence recorded in the regional corporation of Sangre Grande and the lowest in the city of Port of Spain. The lack of important information and active surveillance showed that the level of awareness to the disease is low in the country. The disease is still under-reported, and is considered to be of significant public health importance.

608

COMPARISON OF THE KINETICS AND MAGNITUDE OF ANTIBODY RESPONSES AGAINST THE CONSERVED 47 KDA ANTIGEN VERSUS THE VARIABLE 56 KDA ANTIGEN IN SCRUB TYPHUS PATIENTS

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Scrub typhus is an acute, febrile disease caused by the infection of *Orientia tsutsugamushi*. Western blot analysis of whole cell lysates with scrub typhus patient sera has identified at least four protein antigens of *O. tsutsugamushi* with molecular weights of 22-kDa, 47-kDa, 56-kDa and 110-kDa. Almost every clinically diagnosed patient serum reacts with the naturally abundant 56-kDa antigen, but not every patient serum reacts with the less abundant 22-kDa, 47-kDa or 110-kDa antigens. In this study, a total of 438 serial bleedings from 108 patients were investigated for the kinetics and the magnitude of specific antibody responses against the 47-kDa and 56-kDa antigens. Recombinant 47-kDa antigen (r47b from Karp strain) and recombinant 56 kDa antigen (a mixture of three r56s from Karp, Kato, and Gilliam) were used as the antigen in enzyme-linked immunosorbent assay (ELISA). Our results showed that 76% and 93% of these patients had elevated IgM and IgG against r47b respectively, and 98% and 100% against r56s respectively. Two distinct types of anti-r47b and anti-r56s antibody responses were encountered. For the first type of response, the antibodies against r47b antigen appeared about the same time as against r56s antigen. For the second type of response, the antibodies against r47b antigen were induced about two weeks after those against the r56s. This is the first systematic investigation of antibody responses against the conserved 47-kDa antigen versus the variable 56-kDa antigen in scrub typhus patients.

609

PREVALENCE AND RISK FACTORS FOR TRACHOMA IN RWANDA

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Trachoma is the oldest blinding ocular infection which has well known predisposing risk factors necessary for its transmission. The prevalence of trachoma in Rwanda is unknown as no other trachoma population based survey has been undertaken. The aim of this study was to determine the prevalence of Trachoma and assess associated risk factors for transmission of trachoma in Gatsibo and Nyaruguru districts, Rwanda. A population based cross-sectional study of children aged 1 to 9 years and adult women aged 15 and above. Clusters were selected through probability proportion-to- size sampling and eligible persons sampled using a systematic random sampling method. Data collected using 3 generic survey questionnaires (village, household and individual level) as recommended by WHO and analyzed using STATA. 3451 children aged 1 to 9 years and 1841 adult women aged 15 and above were recruited for the study from Gatsibo District (Eastern Province) and Nyaruguru District (Western Province) and underwent ocular examination for trachoma assessment. The prevalence of trachoma follicular (TF) among children aged 1-9 years was 1.32% [95% CI 0.77-1.86] in Gatsibo and 0.73% [95% CI 0.33-1.13] in Nyaruguru Districts respectively; with both districts having a prevalence below the WHO/ITI cut off point of 10% for trachoma to be taken as disease of public health importance. There was no case of blinding Trachoma Trichiasis (TT) and corneal opacity (CO) in both districts. Risk factors for trachoma transmission were minimal. In conclusion, trachoma is not a disease of public health importance in Gatsibo and Nyaruguru districts, Rwanda.

610

A COMPARATIVE HIGH-THROUGHPUT SCREEN OF 300,000 COMPOUNDS FOR REGULATORS OF THE *SALMONELLA ENTERICA* PHOP REGULON BY THE MOLECULAR LIBRARIES PROBE CENTERS NETWORK

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The Southern Research Specialized Biocontainment Screening Center (part of the NIH Molecular Libraries Probe Production Centers Network (MLPCN)) has expertise in the development and implementation of high-throughput screening (HTS) assays for biosafety level 2 or 3 containment of viruses and bacteria. The MLPCN 300,000 compound library was screened against the PhoP regulon in Salmonella enterica serovars Typhi and Typhimurium. Typhimurium is an important cause of gastroenteritis in humans and typhi causes human typhoid. The PhoP regulon is a major regulator of virulence that also controls the adaptation to Mg2+-limiting environments, and enables Salmonella to determine its presence in intra- or extra-cellular environments and to regulate genes required for entry into or survival within host cells. We have developed a 1536-well microplate HTS assay for the discovery of small molecule inhibitors of the PhoP virulence regulon of S. enterica which may lead to novel strategies to inhibit the intracellular persistence of bacterial infection. We used different recombinant PhoP-activated and PhoP-repressed promoter-GFP reporter fusions to quantify the expression of these reporters in *Salmonella enterica* serovar Typhimurium and Typhi grown in PhoP-inducing and non-inducing conditions, for identifying compounds that specifically inhibit PhoP regulated virulence gene expression. There is significant evidence to suggest that the two serovars differ enough to expect that active compounds from one screen might not correlate 100% with those from the other screen. A complete primary screen comparison would validate the serovar Typhimurium screening model for identifying probes that affect serovar Typhi. For both primary assays, Z values were > 0.7, the coefficient of variance was < 4%, and the S/N > 2. Hit rates were ~0.2%. Counter screens and secondary assays for hit confirmation will follow. This highlighted assay is one example of how the collaborative MLPCN algorithm functions to identify chemical probes for the scientific community, and we invite new participation.

611

ACTIVITY OF SELECTED ANTIMICROBIAL AGENTS, INCLUDING TWO EXPERIMENTAL KETOLIDES, AGAINST LEPTOSPIRA SEROVARS *IN VITRO*

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Leptospirosis is an important but often overlooked zoonotic disease which can cause significant morbidity and mortality. Although *Leptospira* species are susceptible to a wide variety of antimicrobials *in vitro*, rapid diagnosis of leptospirosis is not commonly available. Thus, patients with leptospirosis are often treated empirically for a variety of bacterial infections which present as acute febrile illnesses. A broth microdilution technique using a colorimetric growth indicator (AlamarBlue) was used to determine the antimicrobial susceptibility of 19 different antimicrobial agents, including two experimental ketolides, against 8 *Leptospira* serovars. Leptospires were incubated with serial two-fold dilutions of antimicrobials (concentrations of 32 to 0.016 µg/ml) for 5 days at 30°C prior to determination of MIC. Each combination was tested in triplicate with doxycycline and *L. interrogans* serovar Icterohaemorrhagiae strain RGA used as quality controls. Ceftazidime, CEM-101 (Cempra) and cethromycin (Advanced Life Sciences) produced inhibition of all serovars below the lower limit of testing (<0.016µg/ml). Median (range) MIC for other agents included cefazolin and minocycline, 0.125µg/ml (0.06-1µg/ml); doxycycline, 1 µg/ml (0.5-2µg/ml); cephalexin and colistin were 2µg/ml (2-8µg/ml); gentamicin, tobramycin, amikacin and polymyxin B were 4µg/ml (2-16µg/ml); and fosfomycin and rifampin 16µg/ml (4-32µg/ml). Metronidazole, trimethoprim (TMP), sulfamethoxazole (SMX), TMP/SMX, and vancomycin had no activity against the *Leptospira*. *Leptospira* species are susceptible to a wide range of antimicrobials *in vitro*, although some common antimicrobials such as metronidazole, trimethoprim/sulfamethoxazole and vancomycin have no activity. Ceftazidime, and the experimental ketolides CEM-101 and cethromycin reliably produced MIC below the lower limit of detection.

612

EFFICACY OF FIRST GENERATION CEPHALOSPORINS IN A HAMSTER MODEL OF LEPTOSPIROSIS

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Third generation cephalosporins are commonly used in the treatment of leptospirosis. The efficacy of first-generation cephalosporins in this disease is less well studied. Based on initial *in vitro* evidence demonstrating the susceptibility of *Leptospira interrogans* to cefazolin and cephalexin, the efficacy of these agents was studied in our *in vivo* model. Susceptibility testing of *L. interrogans* serovar Portlandvere to cefazolin and cephalexin was performed by broth microdilution assay. Hamsters were infected intraperitoneally (IP) with 10^5 organisms of *L. interrogans*. On days 2 to 6 after infection, groups of 10 animals received 5, 25, or 50 mg/kg/day of cefazolin or cephalexin IP. Five control animals received no treatment, and 10 animals received doxycycline 5 mg/kg/day IP. Survival was monitored over 21 days. Blood cultures were obtained at the time of death to document spirochetemia. The median MICs for cefazolin and cephalexin were 0.125 µg/ml (range 0.125-1µg/ml) and 2µg/ml (range 1-4 µg/ml), respectively. All untreated animals died by day 8 after infection, while 90% of the doxycycline controls survived to day 21. Survival rates for the 5, 25 and 50 mg/kg study groups were: cefazolin 80%, 100%, and 100%, and cephalexin 30%, 90%, and 100%, respectively. Each treatment group showed improved survival compared to no treatment (P values < 0.01). Only the 5 mg/kg cephalexin dose was associated with a worse outcome than doxycycline (P = 0.01). Blood cultures from all untreated animals were positive, and negative in all doxycycline and all treated animals surviving to day 21. Of the 9 treated animals that died prior to day 21, 5 had positive cultures (cephalexin 5 mg/kg - 2 animals, cefazolin 5 mg/kg - 2 animals, cephalexin 25 mg/kg - 1 animal). In conclusion, in agreement with *in vitro* susceptibility data, cefazolin and cephalexin were shown to be effective in the treatment of leptospirosis in this *in vivo* model. These results support a potential role for first generation cephalosporins as alternative therapies for leptospirosis.

613

EFFICACY OF MINOCYCLINE AND TIGECYCLINE IN A HAMSTER MODEL OF LEPTOSPIROSIS

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Leptospirosis is a widespread zoonotic infection characterized by acute febrile illness. Severely ill patients may require empiric treatment with broad-spectrum antibiotics prior to definitive diagnosis. In vitro studies at our institution have demonstrated the activity of minocycline, and its broad-spectrum glycylcycline derivative, tigecycline, against *Leptospira interrogans*. We evaluated the efficacy of minocycline and tigecycline against leptospirosis in a hamster model. Seventy-five hamsters were

infected with 10^5 organisms of *Leptospira interrogans* serovar Portlandvere by intraperitoneal (IP) injection. On days 2 through 6 after infection, six study groups of 10 hamsters received IP injections of either minocycline (5, 10, or 25 mg/kg/day) or tigecycline (5, 10, or 25 mg/kg/day). Five hamsters remained untreated, and ten hamsters received doxycycline (5 mg/kg/day IP) for five days. All hamsters were monitored at least twice daily following infection, and moribund animals were humanely euthanized. The primary endpoint was survival at 21 days. Blood cultures were obtained from all hamsters at time of death to verify the presence or absence of spirochetemia. By day 9 after infection, all untreated animals were dead. In contrast, 100% survival at day 21 was seen in the doxycycline group, as well as each of the tigecycline and minocycline groups. All study groups showed significantly improved survival compared to the untreated group (P values < 0.01), indistinguishable from the doxycycline group. Blood cultures from 4 of the 5 untreated animals were positive, while those from all treated animals were negative. Minocycline and tigecycline were both active against leptospirosis in this hamster model at a level comparable to the accepted standard treatment, doxycycline. In the absence of doxycycline, minocycline may be considered as an alternative treatment, while a broad-spectrum agent such as tigecycline may be useful in the management of severely ill patients prior to a definitive diagnosis being made.

614

METALLO B LACTAMASE POSITIVE NOSOCOMIAL MDR GRAM NEGATIVE BACTERIA IN A CITY OF A TROPICAL COUNTRY

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Metallo β lactamase (MBL) producing Gram negative bacteria are gradually increasing in all countries throughout the globe, leading to very high morbidity and mortality in hospitalized patients. In this study, prevalence of MBL positive bacteria was observed in Gram negative bacterial isolates obtained from hospitals of Kolkata, India. Gram negative bacteria were collected only from patients with confirmed nosocomial infection mainly from ICCU/ITU. MBL status of all isolates were studied along with their common antibiotic sensitivity patterns. In general 40.2% Gram negative bacteria obtained from different patients, suffering from nosocomial infections, were found MBL positive - *Pseudomonas aeruginosa* was highly positive(51.3%) compared to *Klebsiella pneumoniae* (36.7%) and *Escherichia coli* (28.6%). All of these isolated MBL positive Gram negative bacteria also showed multiple drug resistance (MDR, 45%-54% among different isolates), and all of them were found resistant to Cephalosporins. These strains were also resistant to Chloramphenicol to some extent. MBL positive *Pseudomonas aeruginosa* isolates were resistant to both Imipenem and Meropenem, while *E.coli* isolates were resistant to Meropenem only. MBL positive *Klebsiella* spp were highly sensitive to Imipenem. MBL negative strains showed significantly lower resistance particularly in Cephalosporin group when compared to MBL positive strains. MBL producing *Pseudomonas aeruginosa* isolates showed much higher resistance to Netilmicin (60%) in comparison to MBL non producing *Pseudomonas aeruginosa* (21%). Bacteria produce MBL mostly because of the unrestricted use of antibiotics,especially of carbapenem group. Thus a proper antibiotic policy should be implemented in hospitals of Kolkata, India to check high prevalence of these MBL producing bacteria.

615

ACINETOBACTER SPECIES FROM INVASIVE DISEASE IN RURAL THAILAND

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Bacteria of the Genus *Acinetobacter* have emerged as significant clinical pathogens in hospitalized patients worldwide. Originally viewed as noscomial agents, increasing they are being recovered from apparent community-acquired infections of considerable severity and complicated treatment regimens. Here we describe the clinical microbiology of *Acinetobacter* species recovered from invasive disease in rural Thailand. From 2005-2008, over 50,179 blood cultures were obtained from patients hospitalized in the rural provinces of Sa Kaeo and Nakhon Phanom, Thailand. Suspected *Acinetobacter* isolates were identified by standard methods and screened for susceptibility to amikacin, amoxicillin-clavulanic acid, ampicillin, cefotaxime, cephalothin, gentamicin, imipenem, and co-trimoxazole by disk-diffusion. Of the 173 invasive isolates recovered, 53% were associated with pneumonia, 15% with sepsis, and 32% with other severe disease. Isolates comprised a wide diversity of species, ranging from *A. baumannii*, 49 (28%); *A. junii*, 14 (8%); *A. lwoffi*, 2 (1%); *A. genomospecies 3*, 9 (5%); *A. genomospecies 15*, 1 (<1%), and other *Acinetobacter* species, 98 (57%). Further speciation analysis of the isolates is ongoing. Susceptibility testing revealed considerable resistance to antibiotics commonly prescribed in this setting: 55 (32%) of the isolates were resistant to amikacin, 79 (46%) to amoxicillin-clavulanic acid, 141 (82%) to ampicillin, 108 (62%) to cefotaxime, 68 (39%) to imipenem, 148 (84%) to cephalothin, 51 (30%) to gentamicin, and 82 (47%) to co-trimoxazole. As might be expected, resistance to multiple drugs was considerable, especially in combination with imipenem, a key front-line treatment and further susceptibility testing is underway. Our findings indicate that invasive *Acinetobacter* infections are widespread in this community-based rural setting, comprise a wide range of species in this genetically diverse genus, and have broad resistance to common antibiotics.

616

GENETIC RELATIONSHIPS AMONG THREE FAMILIES OF PLASMIDS IN *RICKETTSIA*

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New rickettsial plasmid sequences were recently obtained by whole genome sequencing of *Rickettsia. amblyommii* GAT3-OV, *R. rhipicephali* CWPP, *R australis* Cutlack, and *R massiliae* AZT80 in a CDC-JGI/LANL collaborative effort. *R amblyommii* has three plasmids while each of the other species have one plasmid. We investigated the unique and conserved features of the three families of plasmids found so far in the genus *Rickettsia*. DNAs were purified from rickettsiae grown in Vero or L929 cells and purifed by Renografin density gradient centrifugation. They were sequenced by both shotgun Sanger and 454 pyrosequencing. Sequence comparisons and annotations were made with MAVID, PipMaker, Open Reading Frame (ORF) finding programs (Glimmer, GetORF), REPUTER, tRNAscan-SE, BLAST and with ClustalW. The sequences and of pRam1, pRam2, pRam3, pRrh1, pRaus1, and pRmaAZ were compared to other available plasmid sequences found in *Rickettsia* (pRF and pRFΔ from *R. felis*, pRak1 from *R. akari*, pRbe1 from *R. bellii* OSU 85-389, pRM from *R. monacensis*, pRma from *R. massiliae* MTU5, and

pRaf from *R. africae*). *Rickettsia* plasmid family pRam1 (18 Kb) contains closely related 15 Kb homologues in *R. massiliae* (pRma, pRmaAZ) and *R. rhipicephali* (pRrh1). Each member of this relatively conserved family had unique rearrangements and Insertion/Deletion (INDEL) sites relative to the others as well as a set of highly homologous ORFs. Genetic variation of pRam1 homologues was also demonstrated in other isolates of *R. amblyommii* and *R. rhipicephali*. The pRam3 (30.9 Kb) family contained the more closely related homologues pRak1 (24.4 Kb) and pRau1 (26.6 Kb). Again numerous genetic rearrangements, INDELs, and conserved ORFs exist among these 3 plasmids. pRam2 (22.8 Kb) is a new member of the very diverse and less highly related pRF, pRFDelta/pRM family (62.8, 39.2 Kb/23.4 Kb) which also includes pRbe1 (48.8 Kb) and pRaf (12.4 Kb). pRam2 and pRam3 were also shown to have polymorphisms in other isolates of *R. amblyommii*. Until recently the genus *Rickettsia* was not thought to harbor plasmids and many species do appear to lack them. Polymorphisms useful for molecular epidemiological investigations appear common in the plasmids. The role and importance of these different plasmids in the pathobiology and different phenotypic characteristics of these isolates is an important field for further investigation.

617

GENOTYPING AND MOLECULAR CHARACTERIZATION OF *GIARDIA INTESTINALIS* ISOLATED FROM THE ORANG ASLI (ABORIGINES) IN MALAYSIA

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Giardia intestinalis was isolated from the Orang Asli (aborigine) at Pos Betau, Pahang, Malaysia in a study to determine the genotypic characterization. Of the 321 fecal specimens collected and examined for Giardia intestinalis by using Trichrome staining method, 76 were positives. Amplicons were produced for 42 of these by amplyfying the gene in the region of the nuclear ribosomal RNA small subunit (SSU rRNA) by using nested PCR. All the amplicons were subsequently sequenced in both directions. Fifteen sequences representing the 42 isolates and six reference sequences representing all known *G. intestinalis* assemblages (A-F) were used in the phylogenetic analysis,(using the neighbor-Joining and maximum parsimony methods) utilizing 140 informative nucleotide positions shown previously to differentiate among genotypes. In both the NJ and MP trees, all 21 *G. intestinalis* sequences grouped together with strong support. The reference assemblage B and *G. intestinalis* isolates proved to be in the same cluster using the NJ method and in the same clade using the the MP method. The trees identified *G. intestinalis* assemblages A and B with predominance of the latter. Thus the predominance of anthroponotic genotypes indicates the possibility of anthroponotic transmission of these protozoa in this aboriginal community.

618

EXPANSION OF METRONIDAZOLE RESISTANCE GENES DURING AMOEBIASIS

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The molecular basis of metronidazole resistance has been examined both in anaerobic bacteria, such as *Bacteroides, Clostridium, Helicobacter* and anaerobic parasitic protests such as *Giardia, Entamoeba*, and Trichomonads. A variety of enzymatic and cellular alterations have been shown to correlate with metronidazole susceptibility in these pathogens; however, a common theme has been revealed. Resistant cells are typically deficient in drug activation. Since *Entamoeba* exists in close association with lumen bacteria, therefore, besides the parasite, the resistance conferred by few resistant bacteria during disease state would dictate the efficacy of the drug. We have observed differential expression of nim genes when the genomic DNA isolated from the stool samples were analysed by RT PCR in patients vs healthy individuals. In order to dissect out the mode of action of the drug in the parasite as well as associated bacteia, we have generated a metronidazole-resistant strain of *E. histolytica* by prolonged exposure of a susceptible axenic strain to metronidazole (20 µM) by pulse chase or continuous dosing with the drug. The effects of the drug on trophozoite ultrastructure as shown by TEM included cell swelling and distorted cell shape, a redistribution of vacuoles, plasma membrane damage and formation of extensive empty areas in the cytoplasm of the parasite. We targeted the molecule pyruvate:ferredoxin oxidoreductase (PFO) a component of the electron transport pathway involved in metronidazole activation that was earlier studied at the biochemical level. Resistant amoebae did not substantially down-regulate pyruvate:ferredoxin oxidoreductase. It is found to be defective in the resistant strain. However, increased expression of iron-containing superoxide dismutase (Fe-SOD) and peroxiredoxin were observed. These results strongly suggest that peroxiredoxin and, in particular, Fe-SOD together with expansion of nim genes perhaps in gut associated bacteria are involved in the mechanism of metronidazole resistance during amoebiasis.

619

THE EFFECT OF LOW TEMPERATURE ON EXCYSTMENT OF PATHOGENIC AND NON-PATHOGENIC SPECIES *ACANTHAMOEBA* AND THEIR ABILITY TO UNDERGO FEEDING FRENZY AND CANNIBALISM

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Acanthamoebae are among the most prevalent protists in the environment having been isolated from soil, dust, air, bottled water, seawater, swimming pools, sewage, hospital dialysis units, eyewash stations, contact lens solution, and lens cases. Free-living Acanthamoebae are found in aquatic biofilms. Acanthamoebae exist in both the trophozoite feeding stage and the highly resistant and dormant cyst stage. Trophozoites of *Acanthamoeba* spp. transform into the highly protective cyst stage when placed in adverse conditions. Laboratory experiments have demonstrated that changes in temperature, pH, salinity, or nutrient content trigger cyst formation. In this study we examined the effect of decreasing the temperature from 18 °C to -2 °C on excystment and on the activity of trophozoites of *Acanthamoeba* spp. For our experiments we used non-pathogenic *A. astronyxis*, semi-pathogenic *A. castellanii*, and highly pathogenic *A. culbertsoni*. Initially, we examined what happened to cysts of *Acanthamoeba* spp. when environmental temperatures approached -2 °C and we examined mechanisms involved when dormant cysts transform into actively feeding trophozoites at low temperatures. We also examined the trophozoite feeding behaviors on pathogenic bacteria at low and almost freezing temperatures. We found that *A. culbertsoni*, which is a human pathogen that grows optimally at 37°C, will transform from the cyst to the trophozoite stage as the temperature is lowered from 18 °C to -2°C. Both *A. castellanii* and *A. astronyxis* had peak transformation from cysts to trophozoites at 8°C and 5°C, respectively. *A. castellanii* and *A. astronyxis* grew favorably at 25 °C. When temperatures remained at -2°C for 72 hours, trophozoites of all studied species became cysts. These data suggest as temperature decreases below 18 °C towards freezing, cysts of Acanthamoebae become trophozoites that actively feed, phagocytose bacterial cells, and cannibalize each other in a feeding frenzy. We hypothesize that this feeding frenzy represents an attempt by Acanthamoebae to get one last chance to intake nutrients prior to encystment at temperatures approaching freezing. These data represent new information about the life cycle and feeding behaviors of three species of *Acanthamoeba*, and may help to explain how *Acanthamoeba* spp. can feed in nature as temperatures approach freezing.

620

MYELOPEROXIDASE ADHERES TO AND DESTROYS *ENTAMOEBA HISTOLYTICA* TROPHOZOITES

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Entamoeba histolytica causes colitis and liver abscess in susceptible individuals. The innate immune response plays an important role in the host defense against an amebic invasion. Although neutrophils are the first cell of the immune system to interact with an invading ameba as well as the effector cells that control amebic infection, the mechanisms through which these cells destroy the ameba are still under study. It is known that neutrophils release toxic substances such as serine proteases, superoxides and myeloperoxidase (MPO), which are potentially able to destroy *E. histolytica* trophozoites. For instance, the MPO mediated microbicidal system operates in the interstitial fluid where it may kill amebas. The present study identified an MPO enzyme, which was isolated from nonspecifically stimulated peritoneal exudates of hamster and purified by chromatography using a DEAE-Sepharose and Sepharose 4B-CL column. The purified MPO was subjected to SDS-PAGE and analyzed by Western blot with a polyclonal anti-human MPO antibody. MPO binding to *E. histolytica* trophozoites was analyzed by an immunocytochemical assay using a rabbit anti-human antibody, and MPO amebicidal activity was assayed by spectrophotometric analysis. The number of viable amebas was reduced in a dose dependent manner when incubated with purified MPO (1 and 10 µg/ml) alone or in combination with H_2O_2. Fixed amebas incubated with MPO and subjected to immunocytochemical techniques exhibited an intense membrane label to MPO. Live *E. histolytica* trophozoites that were interacted with MPO exhibited a label to this enzyme in their cytoplasm as well as showing signs of damage (a loss of characteristic architecture, alterations in the plasma membrane, and big vacuole formation in the cytoplasm). Hence, our results demonstrate the binding of MPO to *E. histolytica* trophozoites and the amebicidal activity of this enzyme.

621

EVALUATION OF STOOL FIXATIVES FOR MOLECULAR DIAGNOSTIC DETECTION OF *GIARDIA INTESTINALIS*, *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA DISPAR*

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Molecular techniques are increasingly used to identify parasites at the species level in diagnostic parasitology. It has been shown that fecal specimens kept in formalin, a standard fixative used for preserving helminth eggs, protozoan cysts, oocysts and trophozoites, are not suitable for molecular diagnostic testing. Non-hazardous commercially available stool fixatives, including those from Meridian Bioscience, Inc. (Zn-PVA, and Ecofix®) and from Medical Chemical Corporation (Unifix-CLR) may work well for routine PCR-based molecular diagnosis. This was demonstrated in stools spiked with oocysts of *Cryptosporidium parvum*, which were positive by PCR after being preserved in these fixatives for several weeks. In the present study, we evaluated the ability of PCR to detect *Giardia intestinalis*, *Entamoeba histolytica* and *E. dispar* in stools specimens fixed in Zn-PVA, Ecofix® and Unifix-CLR for up to 6 months. Stool specimens from patients infected with *Giardia* (N=16) and with *Entamoeba* sp. (N=17) based on microscopic examination, were collected and fixed in Zn-PVA, Ecofix® and Unifix-CLR. Samples from these patients were also collected in tubes with no preservative and were followed as well during the study. Fixed and unfixed samples were stored for up to 6 months at room temperature. DNA was extracted from samples weekly for the first 8 weeks (for *Giardia* and *Entamoeba* spp.) and monthly there after for up to 6 months (for *Entamoeba* spp. only). The extracted DNA samples were tested by real-time PCR using a TaqMan probe assay for detection of *G. intestinalis* and a TaqMan assay with probes specific for *E. histolytica* and *E. dispar*. No differences were observed in the real-time PCR detection between the fixed and unfixed samples. In addition, there were no significant differences in the amplification efficiencies of the samples during the study period. These results indicate that these fixatives might be suitable for molecular detection of protozoan parasites.

622

THE EFFECTS OF SALINITY AND PH ON THE SURVIVAL OF PATHOGENIC AND NON-PATHOGENIC *ACANTHAMOEBA* SPP.

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Acanthamoebae are one of the most common protista in the environment. Of the 23 well known species of *Acanthamoeba* 10 are known to cause infection. Pathogenic species of *Acanthamoeba* cause disease worldwide in both temperate and tropical regions including in North America, Europe, Australia, Africa, and South America. *Acanthamoeba* spp. have been isolated from soil, dust, air, bottled water, seawater, swimming pools, sewage, hospital dialysis unites, eyewash stations, and contact-lens-care solutions and contact-lens cases. Clinically, Acanthamoebae are known to cause granulomatous amoebic encephalitis (GAE) and chronic amoebic keratitis in humans. Under adverse environmental conditions, trophozoites revert to cyst stages, which are resistant to physical, chemical, and radiological damage. In this study we examine the effects of high salt concentrations on the survival of *A. astronyxis* (non-pathogenic) and *A. castellanii* (semi-pathogenic). We also examine the effect of pH on the survival of *A. astronyxis*. Growth of both species in concentrations of NaCl 10X higher (1.2 mg/ml) than that of normal growth medium demonstrated no difference. However, at NaCl concentration 100X higher (12 mg/ml) than that of normal growth medium, A. *castellanii* grew and reproduced at a greater rate than *A. astronyxis*. When cultured at different pH ranges (pH 2 - 12) *A. astronyxis* demonstrated optimal growth and reproduction at pH 6 - 8. Incubation at pH > 9 resulted in killing of trophozoites without cyst formation. Incubation at lower pH resulted in reduced growth and reproduction rates. Growth at high osmolarity is the hallmark of pathogenic Acanthamoebae and directly correlates with the pathogenicity of isolates of *Acanthamoeba* spp. However, the precise mechanism of how pathogenic Acanthamoebae can adapt to higher osmolarity and still maintain metabolic activities remains unknown. Acanthamoebae are able to harbor many bacterial and viral pathogens including *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Francisella tularensis*, Coxackie B3 viruses, and Mimivirus. Investigating the effects of osmolarity and pH may be critical to controlling Acanthamoebae as vectors of pathogenic organisms. In addition, these findings have a significant implication on our ability to distinguish between pathogenic and non-pathogenic isolates.

623

ANTI-PROTOZOAL EFFECTS IN KOREA BLACK GINSENG-TREATED MICE

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Alternative medicines has been focused on the treatment of diseases as well as health care. In particular, the Korea red ginseng is one of the famous herbal remedies all over the world. Excellent effects of the Korea red ginseng have been reported with various medical importance. Recently, the Korea black ginseng is getting focused as a new herbal medicine. However, medical significance of black ginseng as a herbal remedy has not been well understood. Black ginseng is made by hot-steamed and then dried many times (9 times) and contains saponin more than red ginseng. Accordingly, we expected a good effect of black ginseng on the treatment of protozoal infection and investigated on anti-protozoal immune responses after oral administration of black ginseng. *Toxoplasma gondii* is well-known as an intracellular protozoa infecting eukaryotic cells and especially prefers to infect macrophage among immune cells. Although the treatment of *Toxoplasma gondii* is depending on the pyrimethamine-sulfadiazine administration, the drug has been also reported on the side effect to sulfa. To investigate anti-protozoal effects of the black ginseng extract, we analyzed survival time and immune responses in *T. gondii*-infected BALB/c mice. The survival time of *T. gondii*-infected mice after the intake of the black ginseng extract increased about 2.9% compared to untreated control mice. We confirmed the increase of NK cells compared to other immune cells such as T-lymphocytes ($CD4^+$ or $CD8^+$) and macrophages in the spleen of mice intraperitoneally infected with *T. gondii* tachyzoites. Our results suggest that the oral intake of black ginseng induce the increase of innate immunity related with NK cells on *T. gondii* protozoa infection model.

624

TOXOPLASMA GONDII INFECTION INDUCED AUTOPHAGIC CELL DEATH OF HOST CELLS

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Programed cell death are divided into two types; caspase dependent apoptosis and caspase independent autophagy. Autophagy is a protective process for the cell survival and it can also play a role in cell death. Autophagy is morphologically characterized by the formation of autophagic vacuoles (autophagosome) in the cytoplasm and fused with lysosome. The membrane bound LC3 II and Beclin 1 are hallmarks of the autophagy. This experiment was undertaken to investigate the induction of autophagy by intracellular protozoan parasite, *Toxoplasma gondii* (*T. gondii*) and relationship between apoptosis and autophagy in *T. gondii* infection. HeLa cells were infected by *T. gondii* (RH) with the ratio of 1:5 (cell: tachyzoite). The synergistic effect of autophagic change was detected in rapamycin+*T. gondii*-treated cells compared with *T. gondii* infection. LC3 II and Beclin 1 expressions were increased after rapamycin+*T gondii* treatment on 18h and 24h post infection (PI) on the Western blot analysis. Beclin 1 mRNA expression was increased on 18h PI. Inhibitor of autophagy (3MA), pancaspase inhibitor (zVAD), calpain inhibitor (calpeptin) were pretreated before rapamycin+*T. gondii* infection. The expressions of LC3 II and Beclin 1 were suppressed by 3MA treatment. And treatment of pancaspase inhibitor and calpeptin increased Beclin 1 and LC3 II protein profile, respectively. On the ultrastructural observation, several autophagic vacuoles and tachyzoites in parasitophorous vacuoles were co-existed in rapamycin+*T. gondii* infection. Pretreatment of 3MA showed disappearance of autophagic vacuoles without affection of parasitophorous vacuoles. In flow cytometry, *T. gondii* suppressed actinomycin D- induced apoptosis and rapamycin+*T. gondii* treatment did not induce apoptosis. In conclusion, *T. gondii* induced HeLa cell autophagy and treatment of apoptosis inhibitors such as pancaspase inhibitor and calpeptin were increased autophagic changes of *T. gondii* infected cells.

625

CROSSTALK OF MAST CELL WITH VAGINAL EPITHELIAL CELL IN INFLAMMATION CAUSED BY *TRICHOMONAS VAGINALIS*

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Mast cells have been reported to be present predominantly in the vaginal smear of patients infected with *T. vaginalis*. Our previous study described that the mast cells activated with *T. vaginalis* showed increased production of histamine and TNF-α. The excretory-secretory products of *T. vaginalis* induced increased chemotactic activity of RPMC (rat peritoneal mast cells), and showed passive cutaneous anaphylaxis in mice. In trichomoniasis, vaginal epithelial cell (VEC) has been thought to plays roles as immune responsive cell and as adherence site for growth of *Trichomonas vaginalis.* The adherence of *T. vaginalis* to VEC is known to be cytotoxic to VEC, and immunological active materials produced by this host-parasite coincubation may be expected to affect on mast cell placed in lamina propria of vaginal mucosa. In this study, we investigated whether the conditioned medium prepared by coincubation of vaginal epithelial cell with *T. vaginalis* could stimulate mast cell. Trichomonads-conditioned medium (TCM) was prepared from culture supernatants from MS74 stimulated with live *T. vaginalis* for 6 h, compared with culture supernatants of MS74 cultured without trichomonads (CM). When human mast cell line (HMC-1) were incubated with TCM or CM or *T. vaginalis* for 1 h, respectively, β-hexosaminidase release induced by TCM or trichomonads showed significant increase compared with that of CM. Inflammatory cytokines such as IL-8, IL-6 and TNF-α were significantly increased in HMC stimulated with TCM than those with CM. In addition to, HMC showed increased migration to TCM containing IL-8 and MCP-1, examined by chemotaxis assay. Therefore, it is suggested that human mast cell might increase release of β-hexosaminidase, inflammatory cytokines such as IL-8, IL-6 and TNF-α on stimulation with TCM, and migrate to TCM. In conclusion, mast cell may be involved in inflammatory response caused by *T. vaginalis* via crosstalk response with human vaginal epithelium.

626

ANTI-TUMORIGENIC EFFECTS OF *TOXOPLASMA GONDII* LYSATE ANTIGEN ON TUMORS PRODUCED BY SARCOMA-180 AND CT-26 CELLS

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Anti-tumorigenic effects of a protozoan parasite antigen have been studied in *Toxoplasma gondii* (RH)-infected mouse model in relation with immune responses. Although Th1 type cytokines produced by *T. gondii* infection were involved in the anti-tumorigenic effects, it cannot be explained as obvious and direct mechanisms. This study was performed to examine the mechanisms of anti-tumorigenic effects in terms of reduction of the tumor mass size after a treatment with *T. gondii* lysate antigen (TLA). The tumor mass was produced by an intradermal injection of sarcoma 180 cells or CT-26 cells, colon cancer (Luc+, GFP+) cell lines, with a plain matrigel into BALB/c mice. Anti-tumorigenic effects were examined by the injection of TLA or formalin-fixed *T. gondii* into the tumor mass of mice. The increase of the tumor size and weight almost stopped after the TLA injection. To examine the mechanisms of anti-tumorigenic effects, expressions of CD31 (PECAM-1), NF-κB (p65), and VEGF on tumor

mass and immune responses were examined. Our results show that levels of CD31 (PECAM-1) and NF-κB on tumor mass were lower in TLA-treated mice than the control mice, suggesting an anti-angiogenesis result of TLA-treated mice. To examine the difference of immune responses between mice with or without TLA injection, the spleen cells were examined on the capacity of cell proliferation and ratios of T cells ($CD4^+$- and $CD8^+$-T cells), macrophages, and NK cells. Mice of TLA and formalin-fixed *T. gondii* infection group were low on the capacity of cell proliferation to Con A-stimulation but increased on the ratio of T cells and NK cells compared to control mice. This result suggested that although the immune responses of tumor bearing mice were suppressed by tumorigenic effects or *T. gondii* infection, TLA injection induced the changes of the immune response on tumor-bearing mice and concomitantly anti-tumorigenic effects by anti-angiogenesis. This result suggests that the injection of TLA is directly related to the reduction of the tumor mass in our sarcoma model.

627

DIAGNOSTIC PARASITOLOGY TRAINING: CDC DPDX TRAINING PROJECT 2006-2008

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Today, factors such as global travel and ecotourism play a crucial role in the emergence of infectious parasitic diseases. Expertise in the identification of parasites in clinical specimens is essential for diagnosis and clinical care. In 1998, the CDC's Division of Parasitic Diseases (DPD) introduced the DPDx project, which includes a website for training and reference diagnosis (www.dpd.cdc.gov/dpdx). Through DPDx, online continuing education courses have been created as well as domestic and international laboratory workshops for intestinal and bloodborne parasites. DPDx support has come from the Food Safety Initiative (FSI), the United States President's Plan for Emergency AIDS Relief (PEPFAR), and the President's Malaria Initiative (PMI) with the collaboration of the National Laboratory Training Network (NLTN) and the Association of Public Health Laboratories (APHL). Workshops were customized according to questionnaires given to laboratorians and included lecture as well as hands-on laboratory time. We reviewed the past 3 years (2006 to 2008) of pre- and post-test data accumulated from 6 intestinal and 6 bloodborne parasite workshops to determine how effectively participants learned the material presented. All but one (83%) of bloodborne workshops showed significantly higher post-test scores in comparison to pre-test scores. With intestinal workshops, only 2 out of 6 (.33%) workshops showed significantly higher post vs pre test scores. Although the small number of workshops sampled precludes concluding an overall statistical difference in subject specific workshops, the data suggests that the impact of the bloodborne workshops may be greater than the intestinal workshops. This may be due to the audience having less skills or knowledge on the identification of bloodborne parasitic pathogens prior to the workshops, in comparison to intestinal parasites (pre-score mean = 45 vs 62 respectively). This analysis will serve to redirect the training objectives of future DPDx workshops.

628

ACANTHAMOEBA KERATITIS IN A PREGNANT CONTACT LENS WEARER IN TURKEY

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Acanthamoeba species can cause granulamatous amebic encephalitis and chronic, progressive amebic keratitis. Amebic keratitis is most commonly mistaken for stromal herpes keratitis. Contact lens wearers are most at risk for this infection. Diagnosis of the disease is difficult and infection can result in blindness of the affected eye. We report a case of amebic keratitis in a 42-year-old three months pregnant woman who was a regular contact lens wearer. She was admitted to ophthalmology department with pain, foreign body sensation and redness of her left eye. Dendritic epithelial keratitis was observed. The patient's corrected visual acuity was 20/60 in left eye. Direct smears, cultures, special stained preparations of cornea and both lenses were reported as negative. Also, serological tests were negative for herpes simplex virus 1 and 2. She was treated for a possible bacterial/viral keratitis using topical tobramcyin drops and acyclovir ointment. Following epithelial healing, stromal edema developed in left eye after one month. She was referred to a corneal specialist and herpetic disciform keratitis was diagnosed. She was treated using subconjunctival triamcinolone acetonide injection, topical prednisolone acetate drops, topical atropine drops and acyclovir ointment. Three months later the visual acuity dropped to light perception. Due to dense scarring, penetrating keratoplasty was performed after delivery and patient's visual acuity improved to 20/100. However, the visual acuity again dropped to light perception in one month with recurrent keratitis and scleritis. Culture and the hematoxylin-eosin stain of scrapings from cornea, conjunctiva from scleritis area and tenon layer excision revealed *Acanthamoeba* spp. Then, the therapy was altered to topical chlorhexidine 0.02%, polymyxin B sulfate drops and topical promadine isethionate, along with oral ketoconazole 200 mg twice daily. After therapy, symptoms of uveitis and scleritis regressed but keratitis became more severe. Corneal graft deteriorated with hazy stromal infiltration and epithelial defect. Consequently, enucleation of her left eye was performed due to intractable pain. This is the first case of severe keratitis caused by *Acanthamoeba* to be reported from Turkey and demonstrates that this emerging pathogen can be a cause of severe keratitis in pregnant women.

629

SENSITIVE MULTIPLEX PCR ASSAY FOR *GIARDIA* AND *CRYPTOSPORIDIUM* USING DNA CAPTURE

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Giardia and *Cryptosporidium* are significant outbreak-associated human enteropathogens. Diagnosis is conventionally performed by microscopy or immunoassay. Several nucleic acid amplification tests have been reported, however PCR on stool extracts is complicated by low target template concentrations and PCR inhibitors. In this work we developed a multiplex PCR assay for *Giardia* and *Cryptosporidium* that exhibited similar sensitivity than the singleplex assays. We then increased the lower limit of PCR detection in stool samples ~100 fold through a DNA extraction procedure that incorporated 4 target specific capture oligonucleotides. This DNA capture-multiplex PCR protocol offered greater sensitivity than ELISA on artificially spiked specimens and was validated on clinical stool specimens from Tanzania. This work underscores the importance of DNA extraction

for PCR of enteropathogens and provides a highly sensitive protocol to detect *Giardia* and *Cryptosporidium*.

630

A LOW-PROTEIN MALNUTRITION MODEL OF CRYPTOSPORIDIAL INFECTION IN WEANED MICE

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Cryptosporidiosis has been shown to have short- and long-term impacts on growth and development of children. Animal models are needed that mimic the effects in humans, and thus can be used to test the efficacy of vaccines and drugs to prevent and treat infection. A model has been developed in neonatal mice, but to date no model has been developed for weaned mice with a more fully functional immune response. Our goal is to outline a new model of cryptosporidial infection that correlates more closely to the complex interaction between the immune response and infectious agents in children transitioning from breast-feeding to solid foods. Weaned 21-day old mice were fed a standard diet for 3 days to allow for normal intestinal adaptation. On day 24, some (n=42) received a feed containing 2% protein and some (n=16) received an isocaloric, 20% protein feed. During a 12-day feeding period, the diet and water were given *ad libitum* and the weight of each mouse was recorded daily. On day 36, both cohorts were divided into infected (n=8 for nourished, n=22 for malnourished) and uninfected controls (n=8 and n=20, respectively). Oocysts were excysted prior to infection in a 20% bleach solution. Infected mice received an inoculum of $5x10^7$ *C. parvum* oocysts by oral gavage in 75 µl of solution; controls received 75 µl PBS alone. Weights were recorded through day 50 and stools were collected for oocyst shedding counts by qPCR. Protein deprivation led to severe growth shortfalls during the feeding phase. By the end of the infection phase, malnourished infected mice exhibited additional growth shortfalls, lagging uninfected malnourished controls by 15%. Malnourished infected mice also demonstrated much higher oocyst stool shedding compared to nourished infected mice, maintaining a 10 to 100-fold increase over time even as the infection tapered in both groups. This model is also being used in on-going studies to compare the efficacy of nitazoxanide-like compounds synthesized by our group. In conclusion, in the low-protein weaned murine model, malnutrition intensified cryptosporidial infection, while infection further impaired normal growth, suggesting the potential use of the model to demonstrate the vicious cycle of malnutrition and enteric infection.

631

SIMULTANEOUS LUMINEX BASED DETECTION OF MULTIPLE ENTEROPATHOGENS - PROTOZOA AND MICROSPORIDIA

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Given the range of potential enteropathogens implicated in causing diarrhea, we sought to detect multiple PCR amplicons in a multiplex Luminex reaction. We have started our work on enteropathogenic protozoa. Specific primers and probes have been designed for the 18s rRNA gene of *Entamoeba histolytica* (X64142), *Isospora belli* (AF106935), *Enterocytozoon bieneusi* (L07123), assemblages A and B of *Giardia intestinalis* (AF199446 and U09492), *Encephalitozoon intestinalis* (U09929), *Cyclospora cayetanensis* (AF111183) and the COWP gene for *Cryptosporidium* spp. (AF164102, AF093491, and AF112573). DNA purified from stool (Fujifilm QuickGene DNA tissue kit) enters a three step process consisting of PCR amplification using biotinylated primers, followed by hybridization to amine-modified probes covalently linked to carboxylated spectrally-distinct microspheres, followed by addition of streptavidin PE to detect specifically-bound amplicon. Luminex results are reported as microsphere-specific mean fluorescence intensity (cMFI) normalized to background. Singleplex PCR reactions of stool extracted samples infected with *Cryptosporidium* spp., *C. cayetanensis, G. intestinalis*, *E. bieneusi*, *E. histoltyica*, *E. intestinalis,* and *I. belli* yield high detection calls (e.g., cMFIs of 77.3, 119.4, 41.6, 59.0, 81.0, 71.5, and 53.2, respectively). We have converted the assays to multiplex: a 5-plex reaction for *Cryptosporidium* spp., *C. cayetanensis*, *E. intestinalis*, *E. histolytica* and *I. belli (*cMFIs of 28.3, 42.3, 10.3, 12.9 and 26.7) and a 2-plex reaction for *G. intestinalis* and *E. bieneusi* (cMFIs of 15.6 and 21.6). The assay is currently being validated using patient diarrhea samples from Bangladesh and Tanzania and are expanding the assay to incorporate bacterial, viral, and helminthic enteropathogens.

632

EFFECTS OF *TOXOPLASMA GONDII* INFECTION ON THE PROGRESS OF EXPERIMENTAL ALZHEIMER'S DISEASE IN MICE

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Alzheimer's disease (AD) is the most common cause of dementia in the aged-people and results in a progressive and permanent decrease in the memory and cognitive abilities. AD is characterized by the widespread neuronal degeneration containing synaptic and neuronal loss, extracellular neuritic plaques containing amyloid-β peptide, and intracellular neurofibrillary tangles. Infection of *Toxoplasma gondii*, an intracellular protozoan parasite, was caused by the ingestion of tissue cysts, tachyzoites or oocysts. After the proliferation of tachyzoites in the early acute stage of infection, the parasite forms cysts, which are called bradyzoites, preferentially in the brain and moved to the chronic stage. In this study, we examined the effects of *T. gondii* infection in the brain on the progress of neuronal damages and memory loss in a mouse Alzheimer model. To investigate whether the learning and memory of Tg2576 mice are affected by *T. gondii* infection, the water maze and Y maze test were carried out. In *T. gondii*-infected Tg2576 mice, the time spent in the zone 4 (26.77 sec, platform) was longer than those in other zones (zone 1, 16.36; zone 2, 9.87; zone 3, 6.98 sec) in the water maze test. However, the uninfected Tg2576 mice showed the different pattern in the water maze test (zone 1, 20.63; zone 2, 14.88; zone 3, 7.38; zone 4, 17.1). Furthermore, the results of Y maze test also showed that the infection of *T. gondii* attenuated the spontaneous impairments of memory function in Tg2576 mice. To examine the inhibitory effects of *T. gondii* infection on formation of amyloid plaques, the cortex of the brain was stained with congo red. The amyloid plaques in the cortex of unifected Tg2576 mice were more numerous compared to *T. gondii*-infected or wild type mice. β-amyloid protein was also clearly detected by uninfected Tg2576 mice in western-blot analysis. Our results suggest that the infection of *T. gondii* can attenuate the impairments of memory function by inhibiting the formation of beta-amyloid plaque in a mouse AD model.

633

DRUGS FOR TREATING *SCHISTOSOMA HAEMATOBIUM* AND *S. MANSONI* INFECTIONS

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Schistosomiasis is a common parasitic disease, yet often neglected. Guidelines recommend praziquantel (PZQ) for the treatment and control of schistosomiasis, with no real alternative. Metrifonate was widely

used against *Schistosoma haematobium* and then withdrawn in 2000. Oxamniquine use against *S. mansoni* has declined. Industrial production of both drugs has almost stopped. We conducted two Cochrane systematic reviews and assessed the efficacy and safety of drugs, used alone or in combination, for treating *S. haematobium* and *S. mansoni* infections. We searched MEDLINE, EMBASE, LILACS, conference proceedings and contacted specialists in the field. Regarding drugs for treating *S. haematobium*, the search identified 24 randomised controlled trials, with 6,315 participants. Primary outcome measures were parasitological failure and egg reduction rate. The review confirmed that the standard recommended dose of PZQ (single 40 mg/kg oral dose) is efficacious and safe in treating urinary schistosomiasis. Metrifonate (3 x 7.5-10 mg/kg oral doses administered fortnightly) also shows good therapeutic and safety profile. Evidence on artemisinin derivatives is currently inconclusive. Regarding drugs for treating *S. mansoni*, a total of 46 trials have been identified and data extraction and analysis is ongoing. Most of the trials included in our reviews were insufficiently powered, lacked standardization in assessing and reporting outcomes, and had a number of methodological limitations. We discuss the implications of these findings with respect to public health and research methodology and propose priority research needs.

634

COMPARISON OF TWO COMMERCIALLY AVAILABLE URINE CCA ASSAYS FOR THE DETECTION OF *S. MANSONI* INFECTION IN WESTERN KENYA

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Recently, urine-based antigen capture assays for the detection of schistosomiasis have become commercially available. These assays can distinguish between past and current infections and correlate with infection intensity, but their sensitivity and specificity have not been field tested. As part of a study on *Schistosoma mansoni* infections of children in an area of high transmission in western Kenya, we compared the performance of two different circulating cathodic antigen (CCA) assays available from Rapid Medical Diagnostics (Pretoria, South Africa). One CCA assay is performed in the laboratory and utilizes a standard curve to facilitate evaluation of infection intensity. The other CCA test is a point of contact (POC) assay. CCA assay results were also compared to existing data on schistosome infection levels determined by the Kato-Katz method and an ELISA for anti-schistosome IgG. The laboratory and POC CCA assays suggested that 53.2% and 62.4% of children were positive for schistosomiasis, respectively. These results are comparable to ELISA (61.6%), and indicated significantly more schistosome positive children than did fecal examination of duplicate slides of 3 separate stool samples (36.6%). Latent Class Analysis was used to determine the sensitivities and specificities of the CCA urine assays. The laboratory CCA assay had a sensitivity of 89% and a specificity of 86%. The POC CCA assay had a sensitivity of 96% and a specificity of 73%. Results from both the laboratory and POC CCA assays correlate with egg burden. Infection with soil transmitted helminths did not appear to affect the performance of the urine diagnostic assays. Although urine CCA tests are more expensive than reagents for stool exams, the reduced equipment and training needed for the CCA tests, along with the possibility of diagnosing and treating during a single patient visit with the POC test reduce the cost differential. Our results suggest that in areas of high transmission, urine-based assays for CCA may be valuable tools for screening and mapping of *S. mansoni.*

635

DO ENDOGENOUS ANTI-OXIDANTS OF *SCHISTOSOMA MANSONI* PRIMARY SPOROCYSTS PROTECT AGAINST EXTERNAL OXIDATIVE STRESS?

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Previous studies have shown that proteins released during *in vitro* transformation of the miracidial-to-sporocyst stage contain various anti-oxidant (anti-Ox) enzymes that are hypothesized to serve a protective role against host reactive oxygen species (ROS). Moreover, *in vitro* sporocyst exposure to sublethal H_2O_2 levels stimulates an upregulation in gene expression of thioredoxin peroxidase (TPx), a H_2O_2 scavenging protein. To explore the role of endogenous larval anti-Ox in protection against external oxidative stress, we employed an RNA interference (RNAi) approach to knockdown expression of the several *S. mansoni* genes including TPx, glutathione peroxidase (GPx), glutathione-S-transferase (GST26, GST28) and Cu/Zn superoxide dismutase (SOD). Consistent transcript knockdown, compared to a green fluorescent protein (GFP) double-stranded (ds)RNA control was achieved for all enzymes except one, SOD, which exhibited an increase in steady-state transcript levels upon dsRNA treatment. This result was corroborated by western blot analysis. In followup *in vitro* experiments, sporocysts treated with dsRNA for TPx, GPx, GST26 and GST28 were significantly more sensitive to sublethal H_2O_2 treatments than the GFP control or SOD dsRNA-treated larvae as demonstrated by catalase-sensitive increases in parasite mortality. Moreover, in an *in vitro* cell-mediated cytotoxicity assay, sporocyst killing by susceptible NMRI *Biomphalaria glabrata* hemocytes was significantly increased in TPx, GPx and GST26 dsRNA-treated larvae, compared to those treated with GFP or SOD dsRNA. Results strongly implicate endogenously-expressed larval anti-Ox enzymes as serving a critical role in protection against external oxidative stress.

636

GENERATION AND CHARATERIZATION OF IGM AND IGG MONOCLONAL ANTIBODIES THAT BIND FUCOSYLATED GLYCAN EPITOPES FROM *SCHISTOSOMA MANSONI* AND KEY HOLE LIMPET HEMOCYANIN

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The major antibody responses in humans and animals infected with *Schistosoma mansoni* are directed against glycan epitopes of the parasite's glycoconjugates. To facilitate purification and identification of the antigenic glycans from the parasites, we have used splenocytes from *S. mansoni* infected mice to generate hybridomas that secrete monoclonal antibodies to different antigenic glycans of schistosomes. In this study, we describe the generation and characterization of IgM and IgG monoclonal antibodies, K3A10 and F2D2 respectively, from splenocytes of Swiss Webster mice infected for 6wk and 10 wk respectively with *S. mansoni*. ELISA analysis shows that both K3A10 and F2D2 bind to soluble egg antigens (SEA) of *S. mansoni* in periodate-sensitive fashion, demonstrating that the antigenic epitope recognized by the antibodies are carbohydrate in nature. Interestingly, both K3A10 and F2D2 also bind to glycans from keyhole limpet hemocyanin (KLH). Fucose is a major determinant with the epitope recognized by the F2D2 as the binding of the monoclonal antibody to SEA and KLH is inhibited by free fucose and abolished by treatment of SEA with bovine kidney α-fucosidase. Western blot analysis and immunohistological staining of different *S. mansoni* life cycle stages show that the expression of the F2D2-binding glycan epitope is developmentally regulated. The epitope is highly expressed by the larval stages of *S. mansoni*, including eggs, cercariae and schistosomula, but expression by adult schistosomes is greatly diminished. We have used immobilized F2D2 to affinity purify glycoproteins from SEA and KLH that

bear F2D2-binding glycan epitopes and the purified glycoproteins are currently being analyzed to characterize the glycan structure recognized by monoclonal antibody F2D2.

637

SHORT INTERFERING RNAS, AS WELL AS LONGER DS RNAS, DELIVER GENE SILENCING OF CATHEPSIN D OF *SCHISTOSOMA MANSONI*

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The aspartic protease cathepsin D (Clan AA, Family A1) is expressed in the schistosome gut where it plays an apical role in the digestion of hemoglobin released from ingested erythrocytes. In this report, RNA interference was employed to investigate gene silencing of schistosome cathepsin D using dsRNA and short interfering RNA (siRNA) specific for the cathepsin D transcript. Cultured schistosomules of *Schistosoma mansoni* were exposed by square wave electroporation to double stranded RNA (dsRNA) and to one of three short interfering RNAs (siRNA) specific for discrete sites on the cDNA encoding *S. mansoni* cathepsin D. In particular, the siRNAs targeted nucleotides 188-204 (α), 393-412 (beta) and 959-978 (γ) on the cathepsin D transcript. The α and the γ siRNAs delivered stronger knockdown when compared to the beta siRNA. The effect of the α and γ siRNAs was similar to longer dsRNAs of ~500 and 1,285 bp that spanned much or the entire protease encoding transcript. We are now investigating the utility of the α and γ specific siRNAs in the form of short hairpin transgenes driven by the schistosome U6 gene promoter or the human U6 promoter in the pXL-BAC II vector. These and earlier findings suggest that, given the essential role of schistosome cathepsin D in parasite nutrition, this protease could be developed in translational studies as a target for novel anti-schistosomal interventions.

638

INNATE IMMUNE PRIMING OF ADAPTIVE RESPONSES TO HELMINTH INFECTION

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Schistosomes are intravascular helminths that affect approximately 200 million people throughout the tropics and subtropics. Upon infection, current models suggest that an early Th1 response to schistosome infection is replaced at roughly 6 weeks post infection by a Th2 response that is initiated by egg deposition. However, our data suggest that, in addition to IFN-γ, $CD4^+$ T cells also produce IL-10 in response to worm antigens during early infection. We hypothesize that production of IL-10, an anti-inflammatory cytokine, creates an immunomodulatory milieu permissive for parasite establishment and development. To determine whether natural T regulatory (nTreg) cells are an important source of IL-10, wild type mice were treated with monoclonal antibodies that deplete nTreg cells, including S4B6 (αIL-2) and PC61 (αCD25). nTreg depletion was confirmed by FACS analysis and cytokine production investigated by ELISA. Inhibition of IL-2 signaling in infected mice reduces the population of splenic $CD4^+CD25^+Foxp3^+$ T cells and reduces IL-10 production, suggesting nTreg cells are a potential source of IL-10. To determine whether induction of IL-10 by schistosome worms impedes Th1 effector functions, we performed co-infections with *S. typhimurium.* Preliminary data suggest that the Th2 environment induced by early *S. mansoni* infection may inhibit inflammatory responses and allow for increased bacterial proliferation compared to mice infected with *S. typhimurium* alone. The identity and biological relevance of IL-10 producing $CD4^+$ T cells induced by schistosome worms is under further investigation.

639

MODELING SCHISTOSOMIASIS TRANSMISSION AND CONTROL IN A DISTRIBUTED ENVIRONMENT USING A STRATIFIED WORM BURDEN APPROACH

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Multiple factors affect schistosomiasis transmission and worm burden in a distributed human-snail system, including age, behavioral factors, and environment. A traditional mathematical approach to modeling macroparasite systems evaluates mean worm burden (MWB) for human hosts and concurrent infection prevalence for intermediate snail hosts. However, worm distribution in host populations exhibits overdispersed patterns even among otherwise homogeneous risk groups. In standard modeling, one either ignores overdispersion or accounts for its effects by assuming a negative binomial distribution with an uncertain level of aggregation. This approach leaves many unknowns in the application of models to real world control. We implemented a new modeling approach using stratified human populations (according to their level of worm burden: 0, 1, …, etc) in place of MWB. Our approach offers advantages in that: (i) it naturally accounts for overdispersion without prescribing a distribution; (ii) it accommodates calibration based on human prevalence data based on egg counts; (iii) additional determinants of transmission, such as demographics, environmental/ behavioral change, and control (drug treatments, molluscicide) can be easily accommodated. We applied our model to control interventions for a specific transmission environment (Msambweni region in SE Kenya) with interconnected human and snail sites (4 ponds and 10 villages). Based on human-snail infection and population/contact data collected in 1985 and 2000, we calibrated and validated transmission parameters for our model. Present analysis is focused on hypothetical control programs (mass treatment vs. school-based therapy of different age groups) to predict the effects of treatment frequency and coverage levels toward elimination of transmission. While the approach increases system size by replacing MWB with multiple burden strata, it can be easily manipulated mathematically and computationally. It offers substantial improvement in accuracy of prediction for control programs compared to the standard Macdonald approach.

640

PRELIMINARY FINDINGS OF COST OF DISTRIBUTION STUDY FOR THREE ANTHELMINTHIC DRUGS IN PLATEAU AND NASARAWA STATES, NIGERIA

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The ministry of health in Plateau and Nasarawa States, with the assistance of The Carter Center, has provided mass drug administration (MDA) of three anthelminthic drugs (ALB, IVR, and PZQ) for treatment of lymphatic filariasis, onchocerciasis, and schistosomiasis. In 2008, two separate, stand alone MDAs of IVR+ALB and PZQ were given in 9 local government areas. Direct costs associated with the distribution were collected. In total, 1,916,367 persons were treated with co-administered IVR+ALB and 45,334 persons were treated with PZQ. Direct distribution costs, not including personnel costs, ranged from 0.01- 0.06 USD per person treated for IVR+ALB and from 0.01- 0.09 USD for PZQ. Costs were greater in some cases for PZQ distribution because two methods of MDA were used for PZQ: school based or community based. Economies of scale were achieved for both distributions, but PZQ percapita treatment costs dropped more quickly than IVR+ALB for as yet undetermined reasons. Another interesting observation was an increase of cost of delivery per person in urban areas compared to rural areas. This was attributed to per diem differences in urban areas and higher per diem rates for more trained

personnel demanded by more sophisticated populations served by urban MDA programs. While IVR and ALB are donated, until 2008 PZQ had to be purchased at a cost of USD 0.07/tablet, with an average treatment dose of 2.1, or USD $0.15. However, in 2008, through a donation by E-Merck, PZQ was made available through WHO to the Plateau and Nassarawa program free of charge. In 2009 the ministry of health in Plateau and Nassarawa States is expanding simultaneous co-administration of all three medicines as a "triple drug administration" (TDA). We will report the impact of TDA on treatment costs, which we anticipate will reduce percapita treatment costs by 50%.

641

EXPLORING THE IMPACT OF INFECTION-INDUCED IMMUNITY ON ENDEMIC LEVELS OF *SCHISTOSOMA JAPONICUM* IN HILLY AND MOUNTAINOUS ENVIRONMENTS IN CHINA

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Schistosomiasis has long been a threat to villagers living in hilly and mountainous areas of southwestern China where the intermediate snail host is abundant. In recent years our group has focused on the development and parameterization of a village-level mathematical model of *S. japonicum* transmission that accounts for the role of environmental determinants of transmission intensity in endemic villages in Sichuan Province. Until now, our model has not incorporated acquired immunity for which there is evidence in the endemic areas of the lower Yangtze and suggestive evidence in our own studies. To explore the potential impact of infection-induced immunity, a variant of our earlier model was used to describe the dynamics of transmission. Epidemiologic data from our study area suggested modeling of acquired immunity as a function of history of infection, as discussed by Anderson and May and utilized more recently in modeling filariasis, rather than as a function of age. Field data collected in Xichang County from 2000 to 2002 were used for site-specific parameters, and biological parameters were selected from previously calibrated models. Both analytical and simulation studies show that acquired immunity reduces the level of the endemic equilibrium state. Monte Carlo simulations, reflecting parametric uncertainty, also showed a shortened time to reach the equilibrium point. The inclusion of the immunity effect in the model results in a high degree of correlation between the immunity parameters and the parameter reflecting density-dependent worm establishment effects in the host. While these parameter estimates are being refined by calibrating the model to longitudinal infection/re-infection data, at best they will provide refined hypotheses to be interpreted in the light of immunological plausibility.

642

THE IMPACT OF MOBILITY ON *SCHISTOSOMA JAPONICUM* INFECTION: A CASE-CONTROL STUDY OF INDIVIDUAL-LEVEL INFECTION RISK

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Theoretical modeling of *Schistosoma japonicum* transmission has demonstrated that social phenomena, such as mobility between endemic areas may impact the regional persistence of schistosomiasis. We examined whether mobility was associated with infection in 28 villages in Sichuan province, China. A case-control study was conducted, with *S. japonicum* infection determined using the Kato-Katz and miracidial hatch tests. Based on infection status, 130 cases were matched by village to 462 controls. Participants completed a demographic survey, monthly water contact questionnaires, and monthly questionnaires to ascertain mobility patterns. Mobility was defined as the number of monthly periods in which travel outside of one's own village occurred. Adjusting for within-village correlations and local water contact activity, mobility tended to be protective of infection (OR 0.86, 90% CI: 0.76 - 0.98). Local water contact (measured in natural log of contact hours) was positively associated with infection (OR 1.21, 90% CI: 1.06 - 1.37). The majority of mobility was reported between administrative villages and between counties, while less mobility was reported at small scales (between production groups) and at larger scales (between provinces). Generally, those who were men, higher-educated, and aged 18-39 years reported greater levels of mobility. These findings suggest that infection is driven by local water contacts in high risk locations, and that mobility may act as a protective process for those who frequently spend time away from endemic areas.

643

IDENTIFICATION OF GENES AND PROTEINS DIFFERENTIALLY EXPRESSED IN *SCHISTOSOMA MANSONI* ADULT WORMS TREATED WITH PRAZIQUANTEL

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The most efficient control measure of Schistosomiasis is the treatment of infected individuals with praziquantel (PZQ). The action mechanisms of this drug are not still understood. However, it is believed that the β subunits of the calcium channels can be one of the targets. With the objective of studying the mechanism of action of PZQ on the *Schistosoma mansoni* parasite, we intend to identify genes and proteins that are expressed differentially expressed upon treatment. To reach this goal we have used transcriptomic and proteomic approaches, with DNA microarray and bi-dimensional electrophoresis of proteins (2DE) associated to mass spectrometry (MS). Through these techniques it will be possible to identify and to quantify the expression of a great number of genes and proteins, simultaneously, in our biological systems. Male and females adult worms of the parasite were cultivated in RPMI medium culture, with and without PZQ (0,1ug / ml) for 16 hours. The analysis revealed several genes differentially expressed such as high voltage-gated calcium channel beta subunit, Cathepsin B-like cysteine proteinase precursor, SMDR2 and Glutamate transporter family protein 1. In relation to the analysis of differential protean expression, the obtained extracts of proteins of the treated parasites and not treated with PZQ were quantified and submitted to the separation by electrophoresis 2DE. Two hundred well defined spots were observed in the 2-DE gels and only 1 spot was exclusive of the PZQ treated sample. For quantitative analysis the spot densities ratio between the treated and non-treated samples was performed. Eleven spots from the treated and 18 from the non-treated samples presented this ratio values. Five of these over expressed spots were identified as being, one disulfide isomerase protein; one actin; two GST 28; and one myosin light chain. Afterwards, all the proteins expressed differently of interest will be excised from the gel, digested with tripsin and submitted to analyses by mass spectrometry in order to identify them.

644

SEROLOGIC SURVEILLANCE FOR EQUINE INFLUENZA VIRUS IN MEXICO

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There is limited information on the seroprevalence of influenza A virus in horses in Mexico. Previous studies on the epidemiology of this virus in Mexico have focused primarily on humans and birds. Thus, a serological investigation was performed to estimate the seroprevalence of influenza A virus in horses in three geographically and ecologically distinct regions of Mexico: Nuevo Leon State in northern Mexico, Guerrero State in southern Mexico, and Yucatan State in the Yucatan Peninsula of Mexico. Sera were collected from 417 horses from September 2007 through October 2008. Of these, 114 horses were from northern Mexico, 128 horses were from southern Mexico and 175 horses were from the Yucatan Peninsula. A total of 141 (34%) horses had antibodies to influenza A virus using an epitope-blocking ELISA that detects antibodies to all subtypes of this virus. The seroprevalence for influenza A virus in horses was 22% in southern Mexico, 27% in the Yucatan Peninsula and 58% in northern Mexico. Twenty sera with blocking ELISA antibodies to influenza A virus (taken from horses representing each of the three geographic regions) were further tested by the hemagglutination-inhibition and neuraminidase-inhibition assays. All presented with antibodies to the H3N8 subtype. Taken together, these data indicate influenza A virus is a common cause of infection in horses throughout Mexico.

645

THE INCREASING DISEASE BURDEN OF IMPORTED CHRONIC HEPATITIS B VIRUS INFECTION_UNITED STATES, 1973-2007

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An estimated 25% of individuals chronically infected with hepatitis B virus (HBV) die of late complications including cirrhosis and liver cancer. The United States, which implemented a strategy to eliminate HBV transmission through universal immunization in 1991, is a country of low prevalence of chronic HBV infection. There have been approximately 3,000-5,000 new U.S.-acquired chronic HBV infections annually since 2001. Many more chronically infected persons migrate to the U.S. yearly from countries of higher prevalence. Although early identification of HBV infection can help mitigate transmission and late complications, immigrants are not routinely screened for HBV infection at the time of immigration. To estimate the number of imported cases of chronic HBVinfection, we multiplied country-specific HBV infection prevalence estimates by the yearly number of immigrants from each country for 1973-2007. Data were analyzed for trends over 5-year periods. During 1973-2007, 27.2 million immigrants entered the U.S. Sixty-one percent were born in countries of intermediate or high prevalence of HBV infection (range 2%-31%). An estimated 49,500 cases of chronic HBV infection were imported to the U.S. yearly from 2003-2007; without intervention, nearly a quarter of these persons may die of later complications. China, the Philippines, and Vietnam contributed the most imported cases (13.6%, 12.3%, and 11.1%, respectively). Imported cases increased from a low of 91,400 in 1973-1977 to a high of 247,300 in 2003-2007. In conclusion, the yearly number of imported cases of chronic HBV infection exceeds the yearly number of U.S.-acquired cases up to fifteen-fold. Earlier case identification and management of chronically-infected immigrants would strengthen the U.S. strategy to eliminate HBV transmission, and could delay HBV disease progression and prevent some deaths among immigrants.

646

ROLE OF THE MUTATIONS IN E2 PROTEIN IN ADAPTATION OF CHIKUNGUNYA VIRUS TO *AEDES ALBOPICTUS* AND *AE. AEGYPTI* MOSQUITOES

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In 2005-2007 Chikungunya virus caused the largest outbreak/epidemic in documented history affecting parts of Africa, the Indian Ocean islands, India, and Europe. An unusual feature of this epidemic was the involvement of *Aedes albopictus* as a vector. Previously we have demonstrated that a single mutation E1-A226V has significantly changed the ability of the virus to infect and be transmitted by this vector when expressed in the background of well characterized CHIKV strains LR2006 OPY-1 and 37997. In this study we demonstrated that introduction of the E1-A226V mutation into the background of an infectious clone of the Ag41855 strain did not significantly increase infectivity for *Ae. albopictus*. In order to elucidate the genetic determinates which affect CHIKV sensitivity for the E1-A226V mutation in *Ae. albopictus*, genomes of LR2006 OPY-1 and Ag41855 strains were used for constriction of the chimeric viruses and viruses with a specific combination of point mutations in the E2 protein. Based upon the midgut infection rates of the derived virus in *Ae. albopictus* and *Ae. aegypti* mosquitoes, a critical role of the mutations at position E2-60 and E2-211 on vector infection was revealed. The E2-G60D mutation was an important determinant of CHIKV infectivity for both *Ae. albopictus* and *Ae. aegypti,* but only moderately modulated the effect of the E1-A226V mutation in *Ae. albopictus.* However, the effect of the E2-I211T mutation with respect to mosquito infections was much more specific, strongly modifying the effect of the E1-A226V mutation in *Ae. albopictus*. CHIKV infectivity for *Ae. aegypti* was not influenced by the E2-1211T mutation. Distribution of the E2-60G and E2-211I mutations among CHIKV isolates was analyzed revealing high prevalence of E2-211I among CHIKV strains belonging to the Eastern/Central/South African phylogroup. These newly described determinants of CHIKV mosquito infectivity for *Ae. albopictus* and *Ae. aegypti* are of particular importance for the studies aimed at the investigation of the detailed mechanisms of CHIKV adaptations to its vector species.

647

FATAL HUMAN CASES OF VENEZUELAN EQUINE ENCEPHALITIS IN PERU

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Venezuelan Equine encephalitis (VEE) is an emergent disease in the northern Amazon region of Peru. To date, three VEE enzootic subtype viruses have been isolated in Peru: subtype ID, IIIC and IIID. Both subtype ID and IIID are responsible for febrile illness in residents of the Amazon region of Peru; however, subtype ID is the most prevalent circulating virus. Epidemiological studies of febrile illness suggested that the enzootic ID strain circulating in the Amazon region of Peru may be less pathogenic to humans than epizootic variants. In this report, we describe a VEEV subtype ID fatal case with febrile and severe neurological manifestation in a patient living in Yurimaguas, Loreto and a presumptive VEE fatal case in Puerto Maldonado, Madre de Dios. The patient from Yurimaguas, a 7 year-old girl, did not have previous neurological disease and was in good health

condition prior to contracting VEEV infection. Virus titer in the patient sera was similar to titers found in other patients that did not develop neurological complications and survived VEEV infection suggesting that viremia levels do not account for the difference in disease outcome, at least in this case. The second case, a 25 year-old male, presented with clinical manifestations that included headache, fever, muscular ache, nausea, vomiting, diarrhea and epistaxis. The only sample collected from the patient was positive to VEEV based on ELISA IgM (titer 1:6400) and therefore it was considered a presumptive case of VEE. The sample was negative for leptospirosis and other arboviruses. To date, only VEEV subtype ID has been isolated in and around Puerto Maldonado and thus, it is likely that this patient was also infected with VEEV subtype ID. It remains unknown whether host factors and/or secondary infection (such as bacterial infection) may have exacerbated VEEV infection and contributed to the fatal outcome on these cases.

648

PREVALENCE OF TICK-BORNE VIRUSES AMONG PATIENTS WITH UNDIFFERENTIATED FEVER IN BULGARIA

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Tick-borne diseases are not uncommon in Europe. Tick-borne encephalitis virus (TBE) was in the past detected using conventional methods in different parts of Bulgaria. Recently, we reported a cluster of Crimean Congo hemorrhagic fever virus (CCHF) in the south-west of Bulgaria, bordering Greece. Open borders between Bulgaria and other European countries may result in change in incidence and epidemiology of TBE and CCHF. There are no reports in recent literature that describe the actual disease burden in the different provinces in Bulgaria using new diagnostics. In this study, 120 patients with acute febrile illness were enrolled between April 08 and March 09 from 3 hospitals in Sofia (west), Plovdiv (middle) and Burgas (east). Acute and convalescent blood, clinical and epidemiologic data were collected. Sera were tested by IgM ELISA using commercial kits against TBE and CCHF. The overall sero-prevalence was 17.5% (n=21) and 6% (n=7) for TBE and CCHF respectively. Co-infection with both viruses was confirmed serologically in one patient. Sero-positive cases for either virus were more focused on the south and south east of Bulgaria. Male/female sero-positive ratio was 1.4 and 7 for TBE and CCHF, respectively. TBE sero-positive patients presented with nausea, vomiting and skin rash in 50% of the patients, and 24% of total positives, later developed neurologic signs. Most of CCHF sero-positive cases had jaundice (86%); however none had bleeding and all patients recovered. None of the TBE patients indicated consumption of raw milk and half the cases remembered spending time in the forest before disease onset. Detailed clinical presentation and association with other risk factors will be presented. Both TBE and CCHF are under-reported in Bulgaria. Clinical presentation of TBE and CCHF especially in mild cases can be easily confused with other more common pathogens in Bulgaria. This study highlights the importance of including these viruses in the differential diagnosis and warrants further investigations to evaluate the true burden of tick-borne viruses in Bulgaria.

649

FURTHER EVALUATION OF RVF MP12 LIVE-ATTENUATED VACCINE IN CATTLE AND SHEEP IN EGYPT

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The recent outbreaks of Rift Valley Fever (RVF) demonstrate the need to develop an efficient vaccination program MP-12 is a live attenuated vaccine that is potentially more efficacious than the inactivated RVF (IRV) currently in use in Egypt. In this study, we compared the immunologic indices for both vaccines and evaluated the safety of MP-12 in pregnant cows and sheep. Two groups of 8 calves each were vaccinated with MP12 or IRV. The IRV was given in two doses 21 days apart. Blood samples were collected at 7, 14, 21, 28, 56, 180, 360 and 485 days post vaccination (DPV) and tested by ELISA for IgM and IgG and by the PRNT. Gamma interferon was evaluated by *in vitro* stimulation for PBMCs using ELISA. MP12 was administered to 9pregnant cows and 4 pregnant sheep in the first trimester of pregnancy. Animals were kept under observation until parturition and their offspring were followed through 4 months. Viremia was also monitored in 7 heifers vaccinated with MP12 by PCR. Slight fever was recorded in all heifers,6 of 7 heifers were positive by PCR up to 5 DPV. High IgM titers (up to 12,800) were detected in the MP12-vaccinees compared to low titers (up to 400) in the IRV group. IgG antibodies were detectable up to 485 DPV with MP12, while IgG levels waned before 180 DPV in the IRV group. PRNT results indicated high levels of neutralizing antibody throughout the duration of the study in MP-12 vaccinees. All MP-12 vacciinees had detectable antibody by day 14 post-vaccination while the IRV group showed neutralizing antibody beginning at day 28 in 7 of 8 vaccinees. Neutralizing antibody in the IRV group waned much more quickly and was detectable in 4 of 8 animals. Gamma interferon was detected with high OD values only in the MP12 group. None of the vaccinated pregnant cows or sheep aborted or showed any adverse health conditions and their offspring were healthy during the observation period. In conclusion, MP12 vaccine proved to be more immunogenic compared to the local inactivated vaccine and safe for immunization of pregnant sheep and cows.

650

ANTIBODY TO HEPATITIS E VIRUS IN TRAVELERS

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Hepatitis E virus (HEV) is the most common cause of outbreaks of acute hepatitis in lower income countries, with high attack rates and mortality in pregnant women. In higher income countries hepatitis E is a sporadic disease; both foodborne outbreaks and asymptomatic infections have been reported. Among US blood donors, 18-21% have antibody to HEV. Risk for travelers is not well understood. We tested serum samples from a cohort of travelers presenting to clinics in the Boston Area Travel Medicine Network who had lived or traveled outside the US for ≥2 wks to determine prevalence of antibody to HEV. BATMN is a research collaboration of 5 travel clinics in the greater Boston area that care for ~7,500 travelers/yr

in urban and suburban, academic, university-affiliated and independent facilities. IgG and IgM anti-HEV testing was performed at the Laboratory Branch, Division of Viral Hepatitis, Centers for Disease Control and Prevention. Anti-HEV was detected by in-house enzyme immunoassays using as target antigen a mixture comprised of a recombinant mosaic protein containing antigenic determinants of HEV ORF-2 and ORF-3 and a complete ORF-2 baculovirus expressed protein (56K). No data are available about specific exposures or clinical symptoms in this cohort of travelers. Of 162 samples tested, 28/162 (17%) were positive for IgG anti-HEV, 13/162 (8%) for IgM anti-HEV, and 4/162 (2%) for both IgM and IgG anti-HEV. Those positive for IgG and/or IgM anti-HEV were older (mean 48 vs. 39 yrs), more likely to be female (47% vs. 41%), more likely to be born outside the US (58% vs. 57%), and more likely to have traveled for >6 wks (68% vs. 65%) compared to those who were anti-HEV negative, though none of these differences were statistically significant. There was no difference in mean duration of all previous travel (74 days for both groups). Regions of origin of those with antibody to HEV were North America (21), Africa and Latin America (4), and Europe and the Caribbean (2). These rates of anti-HEV in travelers are comparable to those in the general US population. In this cohort of travelers, neither length of stay nor country of origin appeared to correlate with presence of antibody to HEV. A study of seroconversion to anti-HEV after travel and collection of relevant demographic and clinical infomration is underway to help elucidate the role of travel in acquisition of HEV infection.

651

SUSCEPTIBILITY OF *AOTUS NANCYMAAE* OWL MONKEYS TO NORTH AMERICAN AND SOUTH AMERICAN STRAINS OF EASTERN EQUINE ENCEPHALITIS VIRUS

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Eastern equine encephalitis virus (EEEV) is an important human and veterinary pathogen that causes periodic cases of fatal neurological disease. Epidemiological investigations have suggested differences in pathogenesis among the North American (NA) and South American (SA) strains of EEEV. EEEV NA strains are usually associated with neurological/severe disease in humans whereas only 2 fatal human cases have been reported with the EEEV SA variety. Thus, the reduced virulence of SA strains supports the evaluation of these strains in the development of an EEEV live attenuated vaccine. We recently described *Aotus nancymaae* owl monkeys as a suitable model to study EEEV infection. Owl monkeys infected subcutaneously (SC) with an EEEV-NA strain developed measurable viremia and a protective immune response. In this study, we sought to determine the infectivity of an EEEV-SA strain in this non-lethal model after SC inoculation. Animals (n=10) infected with the SA variety developed shorter viremia (1-2 days) than animals infected with the NA variety (3-4 days) and a detectable IgM antibody response beginning on day 5 post-infection (PI). As observed with the NA-infected animals, neutralizing antibodies were detected on day 14 PI. Challenge experiments confirmed the development of 100% protective immunity against both NA and SA strains in the EEEV-SA infected animals. In summary, our study confirmed the value of *A. nancymaae* as an animal model to study EEEV infection as well as the protective response to the virus, and supports the rationale for further testing of SA strains in the development of an EEEV vaccine.

652

PREDICTION, ASSESSMENT OF THE RIFT VALLEY FEVER ACTIVITY IN EAST AND SOUTHERN AFRICA 2006 - 2008 AND POSSIBLE VECTOR CONTROL STRATEGIES

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Historical episodic outbreaks of Rift Valley fever (RVF) since the early 1950s have been associated with cyclical patterns (El Niño and La Niña) of El Niño Southern Oscillation (ENSO) phenomenon which results in elevated and widespread rainfall over the RVF endemic areas of Africa. Using satellite measurements of global and regional elevated sea surface temperatures, and subsequent elevated rainfall and satellite derived-normalized difference vegetation index data, we predicted with lead times of 2- 5 months specific areas where outbreaks of Rift Valley fever in humans and animals were expected and occurred in the Horn of Africa, Sudan and Southern Africa at different time periods from September 2006 to March 2008. Predictions were confirmed by entomological field investigations of virus activity in the areas we identified and by reported cases of RVF in human and livestock populations. This represents the first series of prospective predictions of Rift Valley fever outbreaks and provides a baseline for improved early warning, control, response planning and mitigation into the future.

653

GENETIC CHARACTERIZATION OF A NOVEL HANTAVIRUS STRAIN ASSOCIATED WITH HUMAN ILLNESS IN BOLIVIA

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Hantaviruses are rodent-borne members of the family *Bunyaviridae*. Like all members of this family, hantaviruses have genomes comprised of three negative-sense, single-stranded RNA segments. The three genomic segments S, M, and L code for a nucleocapsid protein (N), two envelope glycoproteins (G1 and G2), and a viral transcriptase, respectively. Hantaviruses have the potential to cause two different types of diseases in humans: in Asia and Europe hantavirus infection is often associated with hemorrhagic fever with renal syndrome (HFRS) while in North and South America hantavirus infection is mostly associated with hantavirus pulmonary syndrome (HPS). From a clinic-based surveillance program of febrile illnesses in Bolivia, we identified a hantavirus from a resident of the Chapare region of Bolivia with symptoms compatible with HPS. We performed RT-PCR on RNA extracted of the whole blood and serum of the patient using primers designed to detect the S segment of New Sigmodontinae-associated hantaviruses. The sequence of the partial S

segment obtained from the nested PCR product was compared with sequences in GenBank; less than 83% nucleotide identity was found with any of the previously-reported New World hantaviruses, included Andes Virus (79.1%), Laguna Negra Virus (79.35%), and Sin Nombre Virus (78.6%). The acute phase sera from the patient was IgM positive to Andes Virus but negative to Laguna Negra Virus and Sin Nombre Virus antigens. Further studies are warranted to determine the reservoirs, ecological range, and public importance of this potentially novel strain of hantavirus.

654

POPULATION-BASED STUDIES TO INVESTIGATE THE EXPANSION OF A NEWLY-INTRODUCED DENGUE VIRUS SEROTYPE IN IQUITOS, PERU

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In 2006, the U.S. Naval Medical Research Center Detachment established a longitudinal cohort in Iquitos, Peru, to study the predictive factors of dengue disease progression and to provide a platform for analyzing dengue virus transmission patterns. Population-based active surveillance for dengue-like disease was established among a cohort of 5,000 participants in 10 zones in urban Iquitos, and six-month serial blood samples were collected from a 2,500 participant subset of the active surveillance population. At the initiation of the study (August 2006), baseline prevalence of neutralizing antibodies was 72% for DENV-1, 68% for DENV-2, 21% for DENV-3, and less than 0.5% for DENV-4; 75% of the population had neutralizing antibodies against two or more serotypes. Over the course of the study, levels of DENV-1 and DENV-2 neutralizing antibodies remained stable, and no DENV-1 or DENV-2 strains were isolated from febrile patients. In contrast, DENV-3 circulation was widespread. By April 2008, approximately 50% of the population had neutralizing antibodies against DENV-3, with 31.5 infections per 100 person-years at-risk (PYR) based on seroconversions between paired samples. During this same period there were 1.6 symptomatic cases per 100 PYR detected through the population-based active surveillance program, suggesting that there are approximately 20 subclinical cases of DENV-3 infection for every symptomatic case in Iquitos. The first active cases of DENV-4 infection in the city were detected in February 2008 through a clinic-based sentinel surveillance program. While no active cases of DENV-4 were detected within the cohort until September 2008, data from the longitudinal arm suggest that DENV-4 was circulating in the population prior to April 2008. By October 2008, DENV-4 had rapidly spread through the cohort population and become predominant DENV serotype in the region. Considering the high levels of subclinical DENV infection observed, longitudinal cohort studies are critical for understanding the dynamics of the introduction and expansion of DENV strains. In addition, these data serve as a baseline for future vaccine trials and vector intervention strategy analyses to be implemented within the cohort.

655

THE USE OF INTRAVAGINAL PRODUCTS AND VAGINAL HYGIENE PRACTICES AMONGST NIGERIAN WOMEN

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The use of vaginal products and vaginal hygiene practices has been linked to the presence of bacterial vaginosis, pelvis inflammatory diseases and could be related to HIV acquisition. These practices have been identified to tighten the vagina thereby increasing sexual pleasure or protect the vagina from infections. This study assessed the effect of intravaginal products and vaginal hygiene in relation to acceptance of Microbicides amongst women in Lagos Metropolis. Three hundred and seventy (370) women who are sexually active and of reproductive age were randomly selected and interviewed using structured questionnaire. Their ages ranged between 19 - 45 years. Each participant completed a questionnaire in order to provide biographical data. Information on use of male/female condom, sexual practices, vaginal hygiene, use of vaginal products and douching habits were collated and analysed. Also, introduction to female Microbicides was implemented through this study. A total of 60% of women accepted the use of vaginal products - gel, herb, vaseline, water to either tighten or lubricate the vagina to increase sexual pleasure while 50% of the women insert herbs before sex to protect themselves from infection and pregnancy since their partner refuses to use condom. For vaginal hygiene, 70% take their bath twice daily during menstruation period (morning and night), 50% bath before sex, 30% clean up with water and soap, 10% with water, others with tissue paper. 20% insert canesten vaginal ovule after menstruation to protect them from vaginal itching. 97% of their sexual partners are not informed of these practices. About 80% of the women are anxiously waiting for introduction of Micribicides in Nigerian market. In conclusion, the success of phase III clinical trial of Microbicides will largely depend on the acceptability of intravaginal product and vaginal hygiene, which is frequently practiced among the women population. This could positively influence the studies of vaginal Microbicides. The understanding of this factor will help in the design, planning and implementation of Microbicides clinical trial in Nigeria.

656

NEIGHBORHOOD WATER AND SANITATION AND DIARRHEAL DISEASE IN AN URBAN AND DEVELOPING REGION OF COASTAL ECUADOR

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Infectious diarrhea remains a significant threat to the health of developing regions. The positive associations between unimproved water and sanitation and diarrhea incidence are well documented. However, few studies have considered the spatial impact of water and sanitation factors and how this may vary across households and neighborhoods. The urban neighborhood is particularly relevant to diarrhea incidence because of the potential for transmission events between houses via person-environment-person transmission (through poor water and sanitation). In this study we characterize the spatial and epidemiological patterns of household diarrhea incidence and their relationship with neighborhood water and sanitation factors in Borbón, a rapidly developing urban area in coastal Ecuador. We use GPS and longitudinal data collected during a series of six, nested case control studies with incidence density sampling to explore the associations between neighborhood water and sanitation factors and the risk of becoming a case household. A case household was identified if at least one household member had three or more loose or watery stools passed in a 24-hour period. Stool samples collected from cases and randomly selected controls were used to estimate pathogen-specific diarrhea (*E.coli*, rotavirus and *Giardia*). Water risk factors included neighborhood practices regarding source, supply, storage and treatment of drinking water. Sanitation was characterized by quality and quantity of sanitation facilities, disposal of sewage and solid waste in the neighborhood. We employed regression methods that capture spatial autocorrelation of outcomes and account for multiple transmission rates to examine the impact of water and sanitation factors in the household's neighborhood on the risk of diarrheal disease incidence. This study is applicable to neighborhood intervention design offering insight on important water and sanitation factors and how the variability in intervention compliance across households may affect diarrheal disease transmission.

657

OUTBREAK OF A *CYCLOSPORA CAYETANENSIS* WITHIN A PERUVIAN MILITARY FACILITY IN LIMA, PERU

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Cyclospora cayetanensis has recently become recognized as an important cause of diarrheal disease worldwide. On February 2009, an outbreak of a diarrheal disease occurred among military personnel in Lima, Peru. Findings from the early cases suggested *C. cayetanensis* as a potential etiologic agent for this outbreak. An investigation was conducted to better define the epidemiology and to identify potential risk factors and sources of infection. We conducted an unmatched case-control study within the affected population. A confirmed case was defined as an individual who presented with diarrhea between January 23rd to March 06th and who was positive for *C. cayetanensis* by direct microscopic observation. A questionnaire was administered to participants to gather demographic, clinical and epidemiological data. Stool specimens were evaluated using direct microscopy for parasites, Kinyoun's acid-fast stain, bacterial culture, and PCR for ETEC heat-labile and heat-stable toxins. Statistical analyses included 95% confidence logistic regression models. We identified 16 cases and 53 controls; 100% were male with a mean age of 19.9+/-1.9 and 20.3+/-2.7, respectively. We observed no difference in mean age between both groups (p=0.48). A 48-hour epidemic curve suggested a point source pattern of infection. The most frequently reported symptoms included diarrhea (100%), abdominal cramps (68.7%) and fever (62.5%) with a mean duration of 6.7 days. We observed an association between history of travel within the 30 days prior to the onset of symptoms (p=0.01) in the univariate analysis. Although not statistically significant, other potential risk factors (i.e., contact with animal excreta, drinking non-purified water etc.) were included in the multivariate analysis (MVA). MVA revealed that only travel was associated with infection (adjusted OR=7.8; 95%IC 1.1 to 56.8), although no one travel destination were associated with illness. Furthermore, no specific meals/food items were associated with the infection. Only one positive culture for *Shigella* was obtained; however, IpaH toxin gene was identified in 8 samples. In conclusion, the only variable significantly associated with infection was a history of travel, most likely as a result of a small sample size. However, additional risk factors were plausible and will be discussed. Additional investigations must be conducted to further elucidate the epidemiology and ecology of this disease.

658

RELATIONSHIP BETWEEN SOCIO-ECONOMIC FACTORS AND TIME-TO-INFECTION WITH *GIARDIA INTESTINALIS* AMONG CHILDREN IN PERU

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Giardia intestinalis infections in otherwise healthy people vary in length, lasting from very few days to several months. It is not clear if exposure factors may be associated with the duration of infections, or if socio-economic variables may play a significant role in these differences. Data from a longitudinal cohort study conducted among 487 young children [1-13 years old] living in Pampas de San Juan, in Lima, Peru were analyzed to test whether specific socio-economic factors contributed to infection, and whether infections of short duration had the same significant predictors as infections of longer duration. Repeated events Cox proportional hazards models were used to evaluate the association between 21 potentially predictor variables and time-to-infection. Analysis was performed for all infections and also when categorized as acute (lasting <14 days), persistent (14-28 days), chronic (>28 days), or long term (lasting ≥14 days) When predictors failed to meet the proportional hazards assumption, extended Cox models were used. All variables were evaluated by univariate and multivariate models. Non-predictor variables ($p \leq 0.05$) were excluded from the full model through backwards elimination. Overall, *Giardia* infections were associated with younger age and variables that indicate low socioeconomic status. Acute infections were associated with low quality hygiene facilities and use of communal cooking. Hazards for long-term (≥ 14 days) giardiasis increased with presence of poultry and having a poor quality roof, and decreased when having more rooms in the house. These findings suggest that better living conditions can reduce the frequency and length of giardiasis in this endemic setting. Future studies should examine further the differences between acute and long-term infections, markers of hygiene and economic development, nutritional status, and incorporate biological or molecular characterization of the parasite.

659

STIMULATION OF MONOCYTES BY FILARIAL EXCRETORY-SECRETORY PRODUCTS: A POTENTIAL ROLE IN MODULATION OF THE LYMPHATIC ENDOTHELIUM?

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Lymphatic filariasis is caused by the parasitic nematodes *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* which infect over 120 million people worldwide. Most microfilaria-positive individuals appear asymptomatic but exhibit subclinical manifestations such as lymphangiectasia. We hypothesized that the excretory-secretory products (ES) of the worms activate the lymphatic endothelium. Initial experiments suggested that ES did not directly activate lymphatic endothelial cells (LEC), leading us to determine whether ES effects are mediated indirectly by other cells types. IL-8, IL-6 and VEGF-A have been shown to support endothelial cell (EC) proliferation and function, so we analyzed the production of these factors by peripheral blood mononuclear cells (PBMC) from naïve donors following stimulation with filarial ES. ES-stimulated PBMCs produced significantly increased levels of IL-8, IL-6 and VEGF-A compared to cells cultured in media alone. Monocytes have been shown to play a role in lymphangiogenesis by secreting soluble factors or by the incorporation of monocytes into the affected vessels; so we hypothesized that monocytes were involved in filarial lymphatic pathology. In order to identify the cell type responsible for the secretion of these EC mitogens in response to ES, naïve CD14+ cells were isolated by magnetic bead separation and analyzed for their ability to secrete IL-8, IL-6 and VEGF-A. CD14+ monocytes are responsible for the production of IL-8 and VEGF-A, but not IL-6. PBMCs from microfilaremic individuals also produced higher levels of spontaneous IL-8, IL-6 and VEGF-A in culture compared to naïve individuals. Interestingly, the percentage of CD14+ cells expressing the lymphatic-specific receptor VEGFR-3 was increased with ES-stimulation; in addition, infected individuals exhibit higher percentages of VEGFR-3+ PBMCs. Collectively, these data suggest that monocytes may support the function of LECs either by the secretion of soluble factors to encourage vessel growth or by the potential direct incorporation of monocytes into the vessels.

660

MACROPHAGE CHOLESTEROL MEDIATES THE ENTRY, PHAGOSOME MATURATION, AND INTRACELLULAR SURVIVAL OF *LEISHMANIA CHAGASI* PARASITES IN A STAGE-SPECIFIC AND VIRULENCE-DEPENDENT MANNER

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Leishmania spp. have a life cycle with two stages: the promastigote, the form inoculated in the skin by the sandfly vector and the amastigote, the disease-causing form of the parasite found in mammalian macrophages. Elimination of the parasite from the infected macrophage requires classical activation generating a pro-inflammatory response. We have shown that *Leishmania chagasi* infection induces a non-inflammatory state in macrophages, and also increases the expression of Caveolin-1 and -3 transcripts, structural components of caveolae. We explored whether caveolae, cholesterol-rich membrane microdomains containing the caveolin proteins, facilitate the entry of *L. chagasi* into murine macrophages. *L. chagasi* co-localized with the caveolae markers ganglioside M1 and Caveolin-1 for up to 48h after phagocytosis. Transient depletion of macrophage membrane cholesterol by exposure to methyl-β-cyclodextrin (MβCD) for 1h, impaired the phagocytosis of virulent, but not of attenuated, *L. chagasi* promastigotes, regardless of opsonization conditions. Pre-treatment with MβCD also increased lysosome fusion 2.7-fold ($P<0.001$), and impaired parasite replication for up to 72h ($P<0.05$), even though macrophage cholesterol was replenished 4h after treatment. Furthermore, side by side experiments showed that promastigote entry was decreased in MβCD-treated macrophages ($P<0.001$), but amastigote entry was not affected. Amastigotes but not promastigotes induced large parasite-containing vacuoles positive for the late endosome marker LAMP-1 4h after infection, suggesting differences in phagosome maturation. Our results support the hypothesis that virulent *L. chagasi* promastigotes exploit a caveolae-mediated pathway to enter host macrophages, and that this cholesterol-enriched entry path may facilitate parasite survival by delaying lysosome fusion until their conversion into amastigotes.

661

DOMINANT ALTERNATIVE MACROPHAGE ACTIVATION IN PROGRESSIVE VISCERAL LEISHMANIASIS IS MEDIATED BY PARASITE-INDUCED STAT6 ACTIVATION AND ARGINASE EXPRESSION

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The clinicopathological features of the hamster model of visceral leishmaniasis (VL) closely mimic active human disease. Studies in humans and hamsters indicate that the inability to control parasite replication in VL could be related to ineffective classical macrophage activation and default toward an alternative activation phenotype. Indeed, the infected hamster spleen showed low NOS2 but high arginase I expression ($p<0.001$) and increased polyamine synthesis ($p<0.05$). Increased arginase activity was also evident in macrophages isolated from the spleens of infected hamsters ($p<0.05$), and arginase I expression was induced by L. donovani in hamster peritoneal macrophages (2.6 fold increase; $p<0.001$) and a hamster fibroblast cell line (BHK) (6.1 fold increase; $p<0.05$). The parasite-induced expression of arginase I was further amplified by exposure of the infected cells to IL-4 ($p<0.05$). Inhibition of arginase activity with norNOHA led to decreased parasite burden in infected macrophages, however we found that norNOHA inhibited parasite arginase and was directly leishmanicidal. Therefore, we used miRNAi mediated selective knockdown of hamster arginase I in BHK cells and found that it led to reduced parasite burden (2.4 fold decrease; $p<0.005$). Since many of the genes involved in alternative macrophage activation are regulated by STAT6, and because the parasite-induced expression of arginase I was independent of exogenous IL-4, we considered the possibility that L. donovani was directly activating STAT6. Indeed, exposure of hamster fibroblasts or macrophages to L. donovani resulted in dose-dependent STAT6 activation ($p<0.05$), even without the addition of exogenous cytokines. Knockdown of hamster STAT6 in BHK cells with miRNAi also resulted in reduced parasite burden (1.6 fold decrease; $p<0.0001$). Collectively these data indicate that L. donovani infection induces macrophage STAT6 activation and arginase I transcription, both of which contribute to alternative macrophage activation and impaired control of infection.

662

PHOSPHATIDYLSERINE EXPOSURE BY AMASTIGOTES OF *LEISHMANIA AMAZONENSIS* IS INDUCED BY HOST IMMUNE RESPONSES

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Leishmania amazonensis (*La*) infection is characterized by an important suppression of the immune response against the pathogen, which in some cases can lead to a severe disseminated disease. We had shown that acting by mimicking an apoptotic cell, amastigote forms are recognized by macrophages through exposed phosphatidylserine (PS) which drives parasite internalization and inactivates macrophage inflammatory activity. Moreover, PS exposure by amastigotes is higher in susceptible mice than in partially-resistant ones which suggests that this feature can be modulated by the infected host. Now we demonstrate that amastigotes obtained from BALB/c or C57BL/6 macrophages display the same amount of PS during the time course of infection, indicating that the PS exposure modulation observed during the *in vivo* infection is determined by interactions between macrophages and other cell types. This hypothesis was confirmed by culturing infected macrophages with lymph node cells from infected BALB/c mice which leads to a higher amount of PS exposure by the purified amastigotes. To further confirm that the immune system activation up-regulate PS exposure by amastigotes we demonstrate that amastigotes derived from immunodeficient BALB/c nude mice display 4-5 times less PS than wild type counterparts. Adoptive transfer of *in vivo* primed $CD4^+$ T cells reverts this result while *in vivo* primed $CD8^+$ T cells do not have any effect. In addition, amastigotes purified from IFNγR KO mice do not expose PS. Those results together indicate that PS exposure by intracellular amastigotes is due to macrophage activation by $CD4^+$ T cells possibly through IFNγ signaling.

663

A RANDOMIZED CLINICAL TRIAL OF THE PROTECTIVE EFFICACY OF TRIMETHOPRIM-SULFAMETHOXAZOLE PROPHYLAXIS AGAINST MALARIA IN HIV-EXPOSED CHILDREN

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Trimethoprim-sulfamethoxazole (TS) prophylaxis is used throughout Africa to prevent opportunistic infections in HIV-infected and HIV-exposed (HIV-uninfected infants born to HIV-infected mothers) infants. Observational studies suggest TS prophylaxis also protects HIV-infected children against malaria. However, data are limited regarding the effect of TS prophylaxis on the risk of malaria in HIV-exposed children. In an area of high malaria transmission in Uganda, we are conducting the first randomized clinical trial of TS protective efficacy against malaria among HIV-exposed infants. 203 HIV-exposed breastfeeding infants were enrolled and prescribed daily TS prophylaxis, per WHO guidelines, until confirmation of PCR-negative HIV status 6 weeks after cessation of breastfeeding. At this point, 185 children were randomized (median age at randomization= 9.6 months) to discontinue or continue TS prophylaxis through age 2 years. 18 HIV-exposed children were not randomized as 10 children were withdrawn prior to randomization and 8 children seroconverted during breastfeeding. Malaria was diagnosed when a child presented with fever and a positive thick blood smear. The association between TS use and malaria incidence was estimated as an incidence rate ratio (IRR) using negative binomial regression. Among 98 infants randomized to continue TS, there were 239 malaria cases after 84.4 person-years (PY) (2.83 cases/PY). Among 87 infants randomized to stop TS, there were 338 malaria cases after 74.5 person-years (4.53 cases/PY). TS prophylaxis yielded a 39% reduction (IRR=0.61, 95%CI= 0.47-0.77, p<0.001) in malaria incidence. We are now following children beyond 2 years of age to compare age-specific malaria incidence between children who stopped TS after breastfeeding (35 cases/6.66 PY= 5.26 cases/PY) and children who stopped TS at age 2 years (18 cases/3.2 PY= 5.63 cases/PY) to evaluate for a post-TS rebound effect on malaria incidence. In our study, TS prophylaxis was modestly protective against malaria in HIV-exposed infants when continued beyond the period of HIV exposure. This degree of protection is substantially lower than previously published in observational studies, possibly due to differences in antifolate resistance, transmission intensity, HIV infection, or other unmeasured confounders.

664

SEVERE DISEASE IN CHILDREN PACKAGE: IMPROVING CARE FOR CHILDREN WITH VERY SEVERE FEBRILE DISEASE PRESENTING TO FIRST-LEVEL HEALTH FACILITIES IN TANZANIA

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Infections that present as very severe febrile disease, such as malaria and pneumonia, are a leading cause of death in children <5 years old (under-5s) in sub-Saharan Africa. According to IMCI guidelines, children 2-59 months who present with very severe febrile disease (fever + general danger sign) should be treated with a parenteral antimalarial, a broad spectrum antibiotic, and referred to a hospital. Overall outcomes for children with very severe febrile disease can be improved by provision of appropriate care. We implemented a multi-faceted intervention, including training, a pediatric medical record, supervision with feedback, and new antimalarials (intramuscular artemether and rectal artesunate) in 43 health facilities in 4 districts in Tanzania. Supervision records were used to evaluate the quality of care before and after training and delivery of new antimalarials. In the first year 20,763 children 2-59 months were seen, including 988 children with very severe febrile disease for whom detailed records were available. After training and delivery of new antimalarials, the proportion of children who received a parenteral antimalarial (88.8% to 98.1%; p=0.03) and a broad spectrum antibiotic (5.0% to 40.2%; p<0.0001) improved. Referral did not change (15.0% to 17.0%; p=0.63). Of 908 patients with very severe febrile disease seen after the new antimalarials were available, 47.4% received intramuscular artemether, 23.9% rectal artesunate, and 27.1% received quinine. In 1422 patients with uncomplicated malaria for whom detailed records were available, intramuscular artemether (n=26; 1.8%) and rectal artesunate (n=17; 1.2%) were rarely used inappropriately as artemisinin monotherapy. In conclusion, multi-faceted intervention was successful in improving care for children with very severe febrile disease; however absolute levels of appropriate care remained low with few patients receiving broad spectrum antibiotics and referral. Uptake of intramuscular artemether and rectal artesunate was adequate with little misuse as monotherapy for treatment of uncomplicated malaria.

665

PREVALENCE OF PFDHFR AND PFDHPS HAPLOTYPES OF GENES ASSOCIATED WITH *PLASMODIUM FALCIPARUM* RESISTANCE TO SULFADOXINE-PYRIMETHAMINE FROM ASYMPTOMATIC ISOLATES IN ANONKOUA-KOUTE (ABIDJAN, IVORY COAST)

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Intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine (IPTp-SP) has been adopted as policy by many countries in sub-Saharan Africa. Unfortunately, because of its low cost and its compliance, the drug is excessively used without indications. To see how drug pressure helps emergence of drug resistant haplotypes, Pfdhps and Pfdhfr were assessed from asymptomatic isolates. The study was performed in Anonkoua-Koute (Abobo-Abidjan), southern Cote d'Ivoire during April and May 2008. Three schools were investigated. Included were asymptomatic children with axillary temperature below 37.5°C with no signs and symptoms of any form of malaria. *Plasmodium falciparum* monospecific infection was detected by thick and thin blood smear. DNA was then extracted from whole blood spotted filter paper by Qiagen method according to the manufacturer's protocol. Extracted DNA was amplified by PCR or Nested-PCR. Sequencing with Big Dye Terminator was then performed followed by an analysis with Sequencher 4.1.4 consisting in comparison of Pfdhfr and Pfdhps sequences from natural strains with the reference sequence from GenBank. A total of 808 children were screened whose age varied from 4 to 15 years old. About 11.9% (n=95) of the children were positive for Plasmodium spp. of which 4.2% (n=4) were P. ovale, 6.3% (n=6) were *P. malariae* and 89.5% (n=85) for *P. falciparum*. We sequenced 48 of all the samples monoinfected with *P. falciparum*. Of this amount, 42 and 28 sequences of Pfdhfr and Pfdhps respectively were analyzable. For Pfdhfr sequences, 14 (33.3%) were wild type NCSI; 15 (35.7%) were triple mutants IRNI. By and large, 19 (45%) samples carried Pfdhfr mutation S108N. Codon position 16 and 164 remained constant without any variation. All of the 28 (100%) samples successfully sequenced for Pfdhps also remained without any variation for codon positions 540 and 581. Except for 2 (7.1%) samples carrying the wild type SAKAA; 5 (17.9%) and 2 (7.1%) were simple mutant AAKAA and SGKAA respectively, and 7 (25%) held the double mutant AGKAA. The haplotypes YGKAA was find but in combination with SAKAS (3.6%) or SGKAS (3.6%) or AAKAA (3.6%) in double infection. Interpretation:

In regions of high endemicity of malaria, incidence of haplotypes carried by Pfdhfr and Pfdhps gives relevant insight on sulfadoxine-pyrimethamine drug pressure.

666

BURDEN OF MALARIA IN PREGNANCY IN WOMEN PRESENTING TO DELIVERY UNITS IN AREAS WITH STABLE AND UNSTABLE MALARIA TRANSMISSION IN CHHATTISGARH, INDIA

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Malaria in pregnancy (MIP) often results in harm to the mother or neonate, though the nature of the adverse effects varies with local patterns of malaria transmission. We conducted a cross-sectional prevalence study at delivery units (DU) in Chhattisgarh state over a 12-month period (2007-2008) in areas of stable (Bastar) and unstable (Rajnandgaon) malaria transmission. In each district two health facilities were chosen, one urban and one rural. Placental and peripheral parasitemia were assessed using both microscopic evaluation of Giemsa-stained thick blood smears and rapid diagnostic testing. 1028 pregnant women were enrolled and screened for malaria. The prevalence of placental parasitaemia was 3.1% (32/1028; 46.8% *Plasmodium vivax* and 53% *P. falciparum*) while only 1.8% had peripheral parasitemia (19/1028; 31.5% *P. vivax* and 68.4% *P. falciparum*). Prevalence was higher among women enrolled in the area with stable as compared to unstable malaria transmission yet the difference was not significant statistically (3.7% vs. 2.9%, p>0.05). There was no difference in prevalence between primigravid vs. multigravid women. The frequency of both placental and peripheral parasitemia was higher among women with fever when compared to asymptomatic subjects (13.6% vs. 2.5%, p<0.0001 and 10.4% vs. 1.2%, p <0.0001 respectively). 30.5% of the pregnant women had untreated bednets in their homes and 32.6% of them had used the net the previous night. Insecticide-treated nets were owned by <1% of households. Of women with parasitemia (either in placental blood or in both placental and peripheral blood), 76.5% had moderate anemia and 11.8% severe anemia. Overall, anemia was more common in women with parasitemia relative to those who were not parasitemic (Hb 9.1 ± 1.9 vs. 10.2 ± 1.6 g/dL, p <0.0001). Among live singleton births, mean birth weight was lower among women with placental malaria vs. those without placental infection (2386 ± 368 vs. 2577 ± 419 g, p<0.0125). Low birth weight was present in 238/930 (25.6%) of pregnancies without placental malaria vs. 14/31 (45.1%) with placental malaria (p = 0.025). In conclusion, there is a relatively small burden of disease due to MIP in women attending DUs in Chhattisgarh. However, given the dense population of this region of India, there is a substantial number of pregnant women at risk for malaria. This suggests the need for enhanced efforts to improve case management and prevention of malaria in this region of India.

667

ROUTINE IRON SUPPLEMENTATION IN HIV-INFECTED PREGNANT WOMEN: IS IT ASSOCIATED WITH THE RISK OF MALARIA PARASITEMIA?

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Iron has been implicated in promoting the replication of malaria parasites. In malaria-endemic areas, routine iron supplementation may increase the malaria incidence and severity. We examined the association between routine iron supplementation (60mg daily) and malaria in HIV-infected pregnant women. From December 2005 to July 2008 we enrolled, in a cross-sectional study, a consecutive sample of 840 HIV-infected pregnant women with gestation ≥34 weeks at Thyolo district hospital antenatal clinic in Malawi. We collected data on socio-demographics, medical history and use of iron supplementation and malaria interventions during pregnancy. Thick blood smears from peripheral blood samples were tested for malaria parasitemia using microscopy. Of the 833 women with information on iron supplementation, 770(92.4%) had ever taken iron supplements during pregnancy. Of the 834 women with microscopy results, 54(6.5%) had malaria parasitemia. Compared to women who had taken iron supplements, those who did not were less likely to have ≥3 antenatal visits (7.9% vs. 61.2%;p<0.0001) and use IPTp (42.9%vs. 88.8%; p<0.0001). The odds of malaria parasitemia in women who did not take iron supplements in pregnancy were 3.62 times (95%CI, 1.77-7.48) the odds of those who did. Among women taking iron supplements, the odds of malaria parasitemia were higher in those taking iron tablets for 31-60 days (OR 1.70;95%CI 0.67-4.26) and for >60 days (OR 1.27:95%CI;0.37-3.94) compared to those taking for 1-30 days adjusting for age, gravidity, bed net use and antenatal visits. However, these were not statistically significant, probably due to inadequate statistical power resulting from low malaria prevalence in the study population. Malaria parasite density among women with >30 days iron intake was higher (median: 2160: IQR; 640-7920 parasites/µl) compared to those with 1-30 days (1620: IQR 120-3560 parasites/µl), though not statistically significant (p=0.18). These preliminary results suggest that iron supplementation should be taken judiciously in HIV-infected pregnant women living in malaria-endemic areas. Because peripheral blood smear microscopy can miss placental parasitemia, we are performing RT-PCR to detect submicroscopic infection. We will also discuss the association between iron supplementation and submicroscopic parasitemia.

668

USING DEMOGRAPHIC SURVEILLANCE SYSTEM TO RECORD EARLY INADVERTENT EXPOSURE OF ANTIMALARIAL DURING PREGNANCY

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The risk of malaria infection and its severity tend to increase during pregnancy and for the same reason WHO recommends the use of antimalarial for case management and intermittent prevention of malaria during pregnancy (IPTp). In sub-Saharan Africa inadvertent exposure of antimalarial in early periods of pregnancy is common and there is no infrastructure to monitor drug safety during pregnancy. Methods. Random sampling of households with women of child bearing age was done during household survey in Ifakara demographic surveillance system

(IDSS) in 2005. Women who were inadvertently exposed to antimalarial in the past two weeks were identified and offered a urinary pregnancy test. Pregnant women who tested positive were followed up to delivery and thereafter mother-child pair followed up quarterly for one year. Overall 268 women were identified and 253 reported use of antimalarial during the past two weeks preceding the interview. Nearly all, 94% agreed to undergo urine pregnancy tests. For those tested, 38 were pregnant and 10 (5%) of all who said are not pregnant before the test were found pregnant after test. There was no evidence of an adverse pregnancy outcome in any woman. Neonatal death was recorded for a birth that happened at home. In conclusion, this assessment has provided a tool to monitor early drug exposure in pregnancy. It has also revealed a gap that exists in morbidity and mortality information available between household and health facility that could be linked to allow proper post birth safety ascertainment.

669

LOW BIRTH WEIGHT, ILLNESS, AND DEATH AMONG YOUNG CHILDREN OF THE KASSENA-NANAKANA DISTRICT OF NORTHERN GHANA: WHO, WHERE, WHEN, AND SOMETIMES, WHY?

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Malaria incidence and severity is changing in many parts of Africa due to increasing use of bednets, antenatal care, and intermittent prevent treatment (IPT) during pregnancy and/or infancy. Interim results from a large birth cohort study in the Kassena Nankana District of northern Ghana may provide indication of the degree to which such measures are being followed and whether significant benefit is derived. From March 2006 until March 2007, newborns (N=2279) were enrolled in a five year, mainly passive, case detection survey. Low birth weight (<2500 g) characterized 18% of enrollments, was associated with being female, and strongly associated with firstborn, and multiple birth status. A point measure of mortality at the end of the 2008 high malaria transmission (wet) season, when children's age ranged from 20-33 months, revealed a significantly higher all-cause death rate (10.3% vs. 4.3%; $p < 0.0001$) among low birth weight (LBW) children. Mean age of death among LBW children was 7.3 months (95% CI: 4.6-10.0) compared with 10.2 months (95% CI: 8.3-12.1) for normal birthweight children. While the majority of underweight births in our cohort were females, males accounted for the majority of LBW deaths (60.5% vs. 39.5%; $P<0.0001$) that occurred. Death was reported for 123 children, only 30% of which occurred in the hospital. Stratified by sector of residence, children from the eastern sector, who made up 23% of the cohort, were under-represented as inpatients, accounting for only 6.4% of the admissions. Severe anemia was significantly more prevalent among these eastern admissions (30/85 = 35.3%) than any other sector (3.0-14%). Since the lowest IPTp coverage and compliance fell within the eastern sector, efforts should be made to ensure timely, reliable, and sufficient supply of drug for pregnant women. Primigravid women and their babies are more vulnerable to malaria and should be targeted for services in the form of ANC, IPTp, bednets, and if possible, delivery in a health clinic.

670

STAGE-SPECIFIC MOLECULAR DETECTION OF *WUCHERERIA BANCROFTI* L3 LARVAE IN MOSQUITOES

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Existing molecular assays for filarial parasite DNA in mosquitoes cannot distinguish between infected mosquitoes that contain any stage of the parasite and infective mosquitoes that harbor third stage larvae (L3) capable of establishing new infections in humans. This study reports the development of a molecular L3-detection assay based on RT-PCR detection of an L3-activated gene transcript in *W. bancrofti* that may be useful to evaluate transmission potential in the context of the Global Program to Eliminate Lymphatic Filariasis (GPELF). Candidate genes were identified by a bioinformatics analysis of EST datasets that focused on cuticle-related genes. Gene expression was assessed by RT-PCR using RNA isolated from mosquitoes collected daily for 14 days after they fed on infected blood. Two cuticlin genes (labeled *cut-1.0* and *cut-1.2*) (Genbank EST Accession # CK854700 and # AF125580, respectively) and one collagen gene (Genbank # CK855471) were considered to be L3-activated because they were first expressed on or after day 9 post-feeding. We developed a multiplex qRT-PCR assay that detects the L3-activated *cut-1.2* transcript and the constitutive transcript *tph-1* (Genbank # U80971). This test simultaneously detects *W. bancrofti* L3 larvae and "any-stage" of the parasite in mosquitoes. It can detect a single infected or infective mosquito in pools of 25 or more. Planned field studies will compare qRT-PCR with dissection for detecting filarial parasites in naturally infected mosquitoes. This general approach (detection of stage-specific gene transcripts from eukaryotic pathogens) may also be useful for detecting infective stages of other vector-borne parasites.

671

THE TRANSCRIPTOME OF *LOA LOA* L3 INFECTIVE LARVAE IN COMPARISON TO THE L3 TRANSCRIPTOMES OF THE OTHER MAJOR HUMAN PATHOGENIC FILARIAE

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Among the major filarial pathogens infective for humans, *Loa loa* is the least well studied and differs from the others in many ways, the most notable being the absence of the *Wolbachia* endosymbiont. To determine the differences between transcripts expressed by *L. loa* and those of other pathogenic filariae, a *L. loa* L3 cDNA library (Ll) was used to generate 3315 expressed sequence tags. ESTs were assembled into non-redundant contigs, which were then assessed for homology to protein and nucleotide databases as well as head-to-head against contig sets assembled from publicly available L3 larval ESTs of *Brugia malayi* (Bm - 5068 ESTs), *O. volvulus* (Ov - 4166 ESTs), and *Wuchereria bancrofti* (Wb - 2048 ESTs). Ribosomal RNA accounted for 189 of 1414 Ll contigs, 23 of 1628 Bm contigs, 14 of 1448 Ov contigs, and 13 of 897 Wb contigs. Using a conservative E-value cutoff of 1E-10, the remaining non-ribosomal contigs could be grouped into only 155 distinct combinations for which homology was shared among all four species. Most abundantly represented among these were transcripts responsible for energy production and metabolism, cytoskeleton and extracellular structures, ribosomal structural proteins, and protein modification and trafficking. Transcripts representing several previously described filarial antigens of unknown function (OvL3, ALT-2, VAH, and MIF-1), were also present in high abundance among all four species. Only two highly related groups had no significant homology to any known functional class. In contrast, functional classes and non-filarial homologs were not identified for many of the 878 *L. loa* contigs

without homologous partners among L3 transcripts of other filarial species. This study provides the first simultaneous and systematic analysis of the L3 larval transcriptomes of these four organisms and identifies the shared set of genes likely to be essential to the L3 larval stage. Further characterization of transcripts unique to *L. loa* may provide important insights into the fundamental biological differences between this and other human pathogenic filariae.

672

DECODING THE INVASION AND MOLTING PROCESSES OF *BRUGIA MALAYI* L3 LARVAE

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Understanding the filarial parasite's transition from the mosquito vector to its human host provides not only insight into the adaptations made by the L3 larvae toward successful invasion and development in the mammalian host but also into the important targets for intervention at this very early stage of filarial development. Using the version 2 filarial microarrays, we have analyzed gene expression profiles at baseline and at 8 time points over a 10 day period (analogous to the lethargus, ecdysis and apolysis stages of the C.elegans molting process) using an established *Brugia malayi* model of molting process (from mosquito L3 to fully molted L4s). Analysis of the gene expression profiles identified >17,000 genes to be up- or down-regulated at any one time point compared to the L3s at baseline. Primary analysis indicated a subset of 268 genes that are induced or repressed throughout the entire molting process beginning at 3 hours at 37oC. These include serpins (BmSPN-1), the vespid venom allergen homologues heat shock proteins, cystatins, thioredoxins and the sheath proteins. Among the cathepsins, known to be important for molting, were CPL-1, CPL-4 and CPL-5 each of which were selectively upregulated compared to the other CPLs. The genes involved in the *Brugia malayi* cuticle formation and organization paralleled those of C.elegans, with additional potential contributions from 218 Wolbachia genes, suggesting that Wolbachia plays an essential role in the developmental process. Ongoing confirmatory studies as well as RNAi targeting key genes should provide definitive answers to those critical and indispensable components of the L3 transition to development in the host.

673

BRUGIA MALAYI GENE EXPRESSION IN RESPONSE TO TARGETED ELIMINATION OF THE *WOLBACHIA* ENDOSYMBIONT

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Brugia malayi, like most human filarial parasite species, harbors an endosymbiotic bacterium of the genus *Wolbachia*. Elimination of the endosymbiont leads to sterilization of the adult female. Previous biochemical and genetic studies have established that communication with its endobacterium is essential for survival of the worm. As a first step in identifying proteins involved in this process, we characterized by microscopy the effects of antibiotic treatment on *Wolbachia* cell structure and on the regulation of *B. malayi* transcripts altered in response to the anti-*Wolbachia* treatment with tetracycline for 7 and 14 days. Using a whole genome microarray, we observe primarily upregulation of *B. malayi* transcripts encoding proteins and enzymes involved in amino acid synthesis and protein translation. These may indicate a generalized stress response induced in *B. malayi* due to a shortage of essential nutrients/ factors which are otherwise supplied by *Wolbachia*. We also found downregulation of transcripts involved in cuticle biosynthesis perhaps reflecting a disruption in the normal embryogenic program. Interestingly, a bimodal pattern of regulation was observed for most of the genes tested further by *in vitro* treatment with tetracycline and quantitative reverse transcriptase PCR. Signaling genes and cysteine proteases were shown to be initially upregulated during the early phase of antibiotic treatment but they were quickly downregulated in the following days, to then be once more upregulated at 4-6 days post-treatment. This pattern may be representative of the worms' response to *Wolbachia* death in different tissues; the early peak reflects potentially the effect of bacteria death on the embryogenic program while the second peak may be a manifestation of the adult worm response to the affected bacteria within the hypodermis. These studies have clearly pointed out few pathways and proteins that are potently involved in the relationships between the endosymbiont and its *B. malayi* host, however testing for relevance during the symbiosis will demand more studies using additional tools for validation.

674

LATERAL GENE TRANSFER IN THE FILARIAL NEMATODES *ONCHOCERCA FLEXUOSA* AND *ACANTHOCHEILONEMA VITEAE* CREATES NOVEL TRANSCRIPTS

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Although lateral gene transfer is appreciated as a powerful driver of genome evolution in many unicellular organisms, transfer of bacterial genes into multicellular eukaryotes is considered unusual. *Wolbachia* endobacteria present in many insect and filarial nematode species represent a special case in which heritable gene transfer is facilitated by infection of the host germline. Most filarial species require *Wolbachia* for development and reproduction, but a few species do not contain these bacteria. Genomic sequencing and bioinformatics analysis showed that the *Wolbachia*-free species *Onchocerca flexuosa* (*Of*) contains at least 90 *Wolbachia*-like DNA sequences, and qRT-PCR studies showed that many of these genes are transcribed. Parallel studies have now shown that a second *Wolbachia*-free species (*Acanthocheilonema viteae* or *Av*), which belongs to a different clade, contains at least 40 *Wolbachia*-like DNA sequences. These findings support the hypothesis that the common ancestor of current filarial species contained *Wolbachia* and that lateral transfer of critically important bacterial sequences made *Wolbachia* redundant and expendable in some species. In both species, most of the transferred sequences have been reduced to short, probably incomplete, fragments by the extensive intron/exon shuffling that has taken place in the nematode genomes. We hypothesize that any sequences that have maintained significant length and intact open reading frames must have only survived because of selective pressure to maintain important biological function. Ongoing studies are designed to create complete inventories of transferred and transcribed *Wolbachia* genes in *Of* and *Av.* The goal of this work is to identify laterally transferred *Wolbachia*-like sequences that are conserved across multiple filarial clades and appear to encode functional proteins. The functions of these genes or domains may give us insight into the biological basis for the dependence of filarial worms on *Wolbachia* endosymbionts.

675

EXPERIMENTAL CONFIRMATION OF FUNCTIONAL OPERONS IN *BRUGIA MALAYI*

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Operons are a common mode of gene organization in *Caenorhabditis elegans*. Similar gene arrangements suggest that functional operons may exist in *Brugia malayi*. To definitively test the hypothesis that functional operons exist in *B. malayi*, we constructed a bicistronic reporter vector consisting of an upstream firefly luciferase gene and a downstream renilla luciferase gene. We surveyed the genome to identify 16 gene pairs that were likely to represent operons. Two of four domains upstream of the 5' most gene from these clusters exhibited promoter activity. When cloned upstream of the firefly reporter in the dual reporter vector, both promoters produced firefly activity, but no renilla activity. When constructs containing the intergenic region of each putative operon cloned downstream of the luciferase gene and upstream of the renilla gene were assayed, neither firefly or renilla activity was detected. However, when constructs replicating the promoter and intergenic arrangement found in the native putative operon were transfected into embryos, both firefly and renilla activities were detected. These data confirm that functional operons exist in *B. malayi*.

676

HIGH SENSITIVITY OF RAPID DIAGNOSTIC TESTS FOR MALARIA IN ROUTINE PATIENT CARE IN RURAL TANZANIA

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Histidine-rich protein II (HRP2)-based malaria rapid diagnostic tests (RDTs) have shown high sensitivity and specificity for detecting *Plasmodium falciparum* malaria in variety of study settings. However, RDTs are sensitive to heat and humidity which may affect their useful life in field settings. We evaluated sensitivity and specificity of RDTs during routine use for malaria case management in peripheral health facilities. From December 2007 to October 2008, HRP2-based ParaHIT® RDTs were introduced in twelve facilities without available microscopy in Rufiji District, Tanzania. Healthcare workers received a single day of instruction on how to perform an RDT and thick blood smear. Job aids, Integrated Management of Childhood Illness guidelines, and national malaria treatment algorithms were reviewed. For quality assurance (QA), thick blood smears for reference microscopy were collected for 2-3 days per week from patients receiving RDTs; microscopy was not routinely performed at the health facilities. Slides were stained and read centrally by a reference microscopist. When RDT and blood smear results were discordant the blood smear was read by two additional reference microscopists blinded to earlier results. Facilities were supervised monthly by the district laboratory supervisor or a member of the study team. 10,650 patients were tested with RDTs. 51.5% (5488/10,650) had a positive test result. Blood smear results were available for 3914 patients, of which 40.1% (1577/3914) were positive for *P. falciparum* malaria. Overall RDT sensitivity was 90.7% (range by facility 85.7-96.5%) and specificity was 73.5% (range 50.0-84.3%). Sensitivity increased with increasing parasite density. In conclusion, successful implementation of RDTs was achieved in peripheral health facilities with adequate training and supervision. QA is essential to the adequate performance of any laboratory test. Centralized staining and microscopic reading of blood smears provided useful monitoring of RDT performance. However, this level of QA is likely not sustainable nationally.

677

A SYSTEMATIC REVIEW OF THE ACCURACY OF RAPID DIAGNOSTIC TESTS FOR MALARIA IN ENDEMIC AREAS

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Rapid diagnostic tests (RDTs) are being increasingly used where good quality microscopy services are not available, as the new first-line malaria drugs (artemisinin-based combination therapies, ACTs) are most cost effective when used following confirmation of malaria parasitaemia. It is therefore important to assess the accuracy of these RDTs in diagnosing symptomatic malaria and the factors that may influence their accuracy. A Cochrane systematic review and meta-analysis including all studies where the same blood samples taken from participants in malaria endemic areas with symptoms of malaria are tested for malaria using both an RDT and an accepted reference standard (microscopy or PCR). We assess the quality of each study using modified QUADAS (Quality Assessment of Studies of Diagnostic Accuracy included in Systematic Reviews) criteria. We present sensitivities and specificities for RDTs compared to reference standards, grouped by test antigen (HRP-2, aldolase and pLDH). We conduct sensitivity analyses using quality components. The review will contain at least 40 studies with assessment of at least 12 commercial RDTs, grouped by types of antigen.

678

ASSESSMENT OF TWO MALARIA RAPID DIAGNOSTIC TESTS, WITH FOLLOW-UP OF POSITIVE PLDH TEST RESULTS, IN A HYPERENDEMIC FALCIPARUM MALARIA AREA

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The most used malaria rapid diagnostic tests (MRDTs) are based on detection of HRP2, e.g. Paracheck-Pf®, but their service is limited in hyperendemic areas by persistent positivity after treatment. PLDH-based tests become negative more quickly, but sensitivity has been reported as falling below the recommended standard of 95%. A new pLDH test, CareStart™ 3-line, claims better sensitivity with continued rapid conversion to negative after treatment. We aimed to 1) compare sensitivity and specificity of CareStart™ to Paracheck-Pf® to diagnose *Plasmodium falciparum* and 2) to assess the time required for true positive CareStart™ tests to become negative. Patients were included if they were aged 2-59 months, presenting at a Médecins Sans Frontières community health centre in eastern Sierra Leone, with suspected malaria defined as fever (axillary temperature > 37.5°C) and/or history of fever in previous 72 hours and no signs of severe disease. Capillary blood was used for the two MRDTs and to perform a blood slide as a reference. All positive patients were treated with AS+AQ treatment (day 0-2). With a positive CareStart™ and negative blood slide, patients were followed from day 2 with repeated CareStart™ and blood slide tests every seven days until CareStart™ became negative or a maximum of 28 days. Sensitivity of Paracheck-Pf® was 98.8% (95% CI 95.8-99.8, 2/169) and of CareStart™, 99.4% (CI 96.8-100.0, 1/169). Specificity of Paracheck-Pf® was 74.7% (CI 67.6-81.0, 44/174) and of CareStart™, 96.0% (CI 91.9-98.4, 7/174) (p<0.001). Of the 155 follow up CareStart™ subjects, 64% (99/155) had a positive test on day 2, 21% (33/155) on day 7, 6% (9/155) on day 14, 2% (3/155) on day 21 and 0.6% (1/155) on day 28. The median time for test negativity was 7 days. Both MRDTs were highly sensitive and met WHO standards for the detection of falciparum malaria. CareStart™ positivity decreased more quickly than Paracheck-Pf® after successful antimalarial treatment, increasing its usefulness in hyperendemic regions where *P. falciparum* is predominant.

679

MISDIAGNOSIS AND OVERTREATMENT OF CLINICAL MALARIA CASES AND THEIR IMPLICATIONS IN WESTERN KENYA HIGHLANDS

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Currently there are intensive malaria control programs being implemented in Africa to reduce malaria burden. Clinical malaria data from hospitals represent available and cost-effective source of infection for evaluating impact of malaria intervention programs. However the reliability of hospital-based data for true malaria incidence is often questioned. Preliminary data on the spatial-temporal distribution of clinical malaria cases through comparison of active and passive case surveillance in western Kenya highlands suggest that clinic-based malaria data does not reflect the true malaria burden in the community. This study seeks to assess the diagnostic accuracy and quality of clinical malaria case management in two health facilities in two districts in western Kenya highlands. Random blood slides were made from patients who were asked by a clinician to go for malaria test in the hospital's laboratory. The result of our slide reading was compared to that of the diagnosis of the technicians in the hospital. Blood slides were also made from patients who were presumptively treated for malaria by symptoms they showed. A questionnaire was designed to collect information from patients and clinicians to determine what contributed to decision on diagnosis of the patient. Data was compiled to measure the accuracy of diagnosis of the clinicians and diagnosis in the lab. Out of the 1042 patients sent by clinicians to the lab for malaria test 92% had fever or history of fever and only 24% had positive malaria slide. Misdiagnosis rate from the hospital lab was found to be 76.4%. Of the 836 patients who were presumptively treated by clinicians with an antimalarial 95% had fever or history of fever and only 36.2% were actually positive for malaria. Mistreatment rate was found to be 66%. Reasons for presumptive treatment in the clinics were varied. The findings show massive over prescription of antimalarials. The implications of these findings are that overtreatment and misdiagnosis will lead to misuse of very expensive combination antimalarial drugs which are currently in use in Africa. The misuse could also trigger early unset of drug resistance to these expensive antimalarial drugs.

680

QUALITY CONTROL OF MALARIA SLIDE DENSITIES

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Various criteria have been used to assess agreement between replicate slide readings of malaria parasite density. Some are based on percent difference between replicates, some on absolute difference, and others on a combination of the two. Criteria which rely on percent differences over-estimate the number of discrepant readings at low parasite densities, while absolute differences have the same problem at high densities. We present a procedure based on the square root of parasite counts. This has a clear theoretical basis, and fewer empirical problems than previous methods. We illustrate the procedure with a dataset of paired readings of asexual parasite density from Tanzania. Each slide was read once at a primary health care (PHC) facility and once at a central laboratory. 528 slides were positive on at least one of these two readings, which were based on 200 white blood cells. On the square root scale, the variation of between-reader differences is approximately constant over the range of parasite densities. Ninety-five percent of the differences in square root counts (PHC minus central) lie between -15.2 and +9.2. These limits of agreement are much wider than the theoretical minimum values, based on the Poisson distribution, which are +/-1.39. The limits can be back-transformed from the square root to the original scale. As expected, the resulting limits of agreement increase with average density. For the Tanzanian data, an average (square mean root) density of 2000 parasites per microlitre (or 50 per 200WBC) has limits of agreement of -8610 to +5220/microlitre (-215 to +131/200WBC). For 10000/microlitre (250/200WBC), the limits of agreement are -19200 to +11700/microlitre (-482 to +292/200WBC). The procedure predicts the gain in repeatability as a function of increased volume read. Applying this approach to other datasets would enable repeatability of parasite density to be compared between studies and help establish target values for quality control.

681

A HIGH-THROUGHPUT POOLING AND TESTING STRATEGY FOR PCR DETECTION AND SPECIATION OF MALARIA DURING PREGNANCY IN KINSHASA

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Pregnancy-associated malaria contributes substantially to the incidence of maternal anemia, low birth weight, and premature birth. We employed a real-time PCR based strategy to detect Plasmodia in 177 pregnant women followed longitudinally from 2005 to 2006 at an antenatal clinic in Kinshasa, Democratic Republic of the Congo, and compared results with microscopy from thick blood smears. At each antenatal visit, thick smears of peripheral blood were prepared and blood spots were collected on filter paper. All women received insecticide-treated bed nets and intermittent preventive therapy with sulfadoxine-pyrimethamine. The blood smears were interpreted on site by trained microscopists. Genomic DNA (gDNA) was extracted from excised blood spots and amplified using a real-time polymerase chain reaction (PCR) assay that targets a sequence of the Plasmodia 18s ribosomal DNA (rDNA) that is conserved across species. Microscopy-positive samples were amplified individually, whereas the microscopy-negative samples were amplified by pooling the gDNA of four samples prior to testing. If a pool was positive, each sample in the pool was then individually tested. All positive individual samples were then speciated using a second real-time PCR assay that targets species-specific sequences of the Plasmodia rDNA of *Plasmodium falciparum*, *P. ovale*, and *P. malariae*. From 182 women, we extracted and amplified 1,268 samples, of which 176 were microscopy-positive (14%). Seventy-three of the microscopy-positive samples were positive by real-time PCR (41%). Of the 1,092 microscopy-negative samples, 35 were real-time PCR positive (3%). In total, we detected 108 parasitemias by real-time PCR, of which 103 were purely *P. falciparum*, 1 was purely P. ovale, 1 was purely *P. malariae*, and 3 were mixed infections with *P. falciparum* and *P. malariae*. Our study highlights both a substantial discordance between microscopic and molecular diagnostics for malaria and the utility of employing a sample pooling strategy for molecular diagnostics in clinical studies.

682

HIGH THROUGHPUT QUANTITATIVE MULTIPLEX 5' NUCLEASE PCR ASSAY FOR DIAGNOSIS AND CLINICAL INVESTIGATION OF MALARIA

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Clinical investigations of malaria are limited by diagnostic tools constrained to small sample numbers and that preclude high-throughput testing, such as standard PCR or real-time PCR that requires analysis of melting curves. We sought to design a real-time quantitative multiplex assay to

differentiate and quantify all *Plasmodium* species infecting humans. We used 5' nuclease (Taqman) quantitative PCR (qPCR) in 96 and 384 well plates, with 18S rRNA gene primers/probes modified from Rougemont et al. with AlleleID software. Since compatible primers/probes could not reliably be identified for *Plasmodium vivax*, we used the *AMA1* gene. Cloned amplicons were used for standard curves. We tested 0.8 to 2 µL of DNA extracted from archived frozen blood from patients with *P. falciparum* (15), *P. vivax (8)*, *P. falciparum/P. vivax* coinfection (2), *P. ovale* (2), and *P. malariae* (1). Blood samples containing bacteria or other hemoprotozoa were tested as controls (16). Analytical sensitivity was linear from 10^7 through 10^0 copies, with a minimum of 10^1 to 10^0 copies (~1-2 parasites or 0.000025% parasitemia). Clinical sensitivity was 94% for *P. falciparum*, 100% for *P. vivax*, and all infections with *P. ovale, P. malariae,* and co-infections were detected. The assay was 100% specific. Mean parasitemia was higher for *P. falciparum* (5.2×10^4 copies) vs. *P. vivax* (2.9×10^3 copies; p=.024). Microscopic parasitemia and qPCR for both *P. falciparum* (R=0.813) and *P. vivax* (R=0.924) were highly correlated. Likewise, a high degree of correlation existed between repeat runs for *P. falciparum* (R=0.975) and *P. vivax* (R=0.998), and when different DNA preparations were made from the same archived samples (R=0.966). Preparation of DNA as single samples or in 96-well plates requires 30 min to 2h, 1h for assay setup, and 1.5h for amplification (total time of < 4.5h); up to 350 samples can be tested simultaneously. Multiplex quantitative PCR for malaria is a rapid, highly sensitive and specific assay that can be used for diagnostic testing or in large clinical studies when high throughput is needed.

683

SARS CORONAVIRUS ADAPTATION TO HUMAN IS PARTIALLY CONSTRAINED BY HOST ALTERATION

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SARS coronavirus (SARS-CoV) was identified as the etiological agent of SARS, and extensive investigations indicated that it originated from an animal source (probably bats) and was recently introduced into the human population via wildlife animals from wet markets in southern China. Virus entry, replication, assembly and release are the main steps of viral life cycle. Proteins involved in each of these steps may undergo adaptive evolution after a virus invades a new host. Previous studies revealed that the spike (S) protein of SARS had experienced adaptive evolution, but whether other functional proteins of SARS have undergone adaptive evolution is not known. We employed several methods to investigate selective pressure among different SARS-CoV groups representing different epidemic periods and hosts. Our results suggest that most functional proteins of SARS-CoV have experienced a stepwise adaptive evolutionary pathway and that different proteins underwent adaptive evolution at different phases of the SARS epidemics. Similar to previous studies, the spike protein underwent strong positive selection in the early and middle phases, and became stabilized in the late phase. In addition, the replicase and assembly proteins were found experiencing positive selection during the late phase. No positive selection was found in any proteins of bat SARS-like CoV. Furthermore, specific amino acid sites that may be the targets of positive selection in each group are identified. This comprehensive evolutionary analysis revealed the stepwise evolution of different functional proteins of SARS-CoVs at different epidemic stages and different hosts. These results support the hypothesis that SARS-CoV originated from bats and that the spill over into civets and humans were more recent events. Furthermore, some amino acid sites may be critical for viral adaptation in different hosts.

684

A COMPARISON OF THE PATHOGENESIS OF CHIKUNGUNYA VIRUS IN MICE AFTER INFECTION BY MOSQUITO BITE OR NEEDLE INOCULATION

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Chikungunya virus (CHIKV) is a mosquito-transmitted *Alphavirus* that causes an illness, characterized by fever, rash and incapacitating joint and muscle pain, which is endemic and sporadically epidemic in Asia and Africa. Due to the widespread distribution of its principal vectors (*Aedes aegypti* and *Ae. albopictus*), CHIKV has the potential to become an important public health problem. The pathogenesis of CHIKV in people is not well understood. To gain a better understanding of CHIKV pathogenesis, a mouse model for CHIKV infection was recently developed in our laboratory. Previous work demonstrated that needle inoculation of CHIKV in young mice causes a self-limiting infection characterized by 3-4 days of viremia and persistence of virus in the skeletal muscle and spleen. Pathological studies indicated severe focal necrosis in the skeletal muscle, with very little pathology observed in other organs. Recent work has shown that the disease course is different if mosquito bite, rather than needle inoculation, is used to infect mice. CHIKV infected *Ae. aegypti* mosquitoes were allowed to feed on young CD-1 mice. Mosquitoes had previously been infected with the same virus stock as used with the needle-inoculated mice. Groups of five mosquitoes were exposed to an anesthized mouse, approximately five days after intrathoracic injection. Subsequently, 3-5 mosquitoes fed on each mouse. In comparison to needle-inoculated young mice, the peak viremia levels in the mosquito-infected mice were 10-fold higher, but approximately 1-2 days shorter. In addition, differences in virus load were also observed in leg muscle and spleen. At day five, only one mouse out of three had detectable virus levels in the leg muscle in the mosquito-infected group; but in the needle-inoculated group at day five, every mouse had greater than 10^4 PFU/mL of virus in the leg muscle. Also in the mosquito-inoculated group, the viral load in the spleen was of shorter duration as compared to the needle-inoculated group. These preliminary results are the first to show a difference in virus titers and tropism between infection by mosquito bite and needle-inoculation of CHIKV in mice. This may be indicative of the importance of the immune response in causing a prolonged infection in the skeletal muscle of CHIKV infected mice. Further studies to understand how the immune response to mosquito bite may alter CHIKV infection in mice are needed.

685

IMMUNOGENICITY AND EFFICACY OF A NOVEL RECOMBINANT VACCINE AGAINST ARGENTINE HEMORRHAGIC FEVER

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Junín virus, a member of the family *Arenaviridae*, causes Argentine hemorrhagic fever (AHF), a zoonotic infectious disease that produces hematological, cardiovascular, renal and/or neurological symptoms in humans. This study was designed to examine the preclinical safety, immunogenicity and efficacy of a recombinant vaccine against Junín virus (TC83/JUNV) in the Hartley guinea pig model, *Cavia porcellus*. The vaccine is based on the genome of TC83 vaccine strain of the Venezuelan equine encephalitis virus (VEEV), which contains Junín virus-specific sequences that express the glycoproteins of the highly pathogenic Romero strain. Twenty female guinea pigs were randomly assigned into four equal groups that received: 1) single immunization with TC83/JUNV; 2) single immunization with TC83 replicon (TC83 control); 3) double immunization

with TC83/JUNV and 4) double immunization with TC83 control; all groups were challenged with the lethal Romero strain of Junín virus. Blood samples were collected from the animals to assess immunogenicity and safety variables. Animals were monitored for clinical signs, body weights, temperatures and survival. Two animals in each group were humanely euthanized on day 10 post-infection and tissue samples were collected for viral load determinations, histopathology and immunohistochemistry. Eight of ten TC83/JUNV immunized animals (80%) developed detectable levels of neutralizing antibodies ($PRNT_{50}$). Thirty-three percent (1/3) of the TC83/JUNV single-immunized animals were protected against lethal Junín virus infection compared to 100% mortality in the TC83 control group. Three TC83/JUNV double-immunized animals (100%) were protected against lethal infection. In conclusion, the results from our pilot study indicate that TC83/JUNV vaccine candidate is immunogenic and efficacious in the guinea pig model. In future experiments, we will test if a higher vaccine dose would allow us to use the single vaccination schedule for induction of full protection in this animal model.

686

MOLECULAR PHYLOGENY OF A NEWFOUND HANTAVIRUS HARBORED BY THE EASTERN MOLE (*SCALOPUS AQUATICUS*)

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Discovery of genetically distinct hantaviruses in shrews (Order Soricomorpha, Family Soricidae), widely distributed in four continents, challenges the long-held view that rodents (Order Rodentia, Family Muridae and Cricetidae) are the primordial reservoir hosts. Recently, novel hantavirus genomes have been detected also in moles (Family Talpidae), including the Japanese shrew mole (*Urotrichus talpoides*), American shrew mole (*Neurotrichus gibbsii*) and European common mole (*Talpa europaea*), suggesting that the evolutionary history of hantaviruses is far more ancient and complex than previously imagined. We now report on the molecular phylogeny of Rockport virus (RKPV), a newly identified hantavirus harbored by the eastern mole (*Scalopus aquaticus*). Hantavirus RNA was detected by RT-PCR in four of five eastern moles, captured in Aransas National Wildlife Refuge in 1986. Newly acquired sequences of the full-length S- and M- and partial L-genomic segments, aligned and compared using ClustalW, indicated low sequence similarity between RKPV and representative rodent- and soricid-borne hantaviruses. Despite the high degree of sequence divergence, however, the predicted secondary structure of the RKPV nucleocapsid protein exhibited the characteristic coiled-coil domains at the amino-terminal end. Phylogenetic analyses, using maximum-likelihood and Bayesian methods, showed that RKPV formed a unique clade, unexpectedly positioned with hantaviruses harbored by rodents in the Subfamily Arvicolinae and Neotominae. Extensive analysis of the coding regions of the RKPV genome, using multiple recombination-detection methods, failed to disclose any evidence of recombination. Distributed widely across much of the eastern sector of the United States, the fossorial eastern mole is sympatric and syntopic with rodent species known to harbor hantaviruses, raising the possibility of multiple host-switching events with local host-specific adaptations. Thus, future in-depth studies of RKPV, viewed within the context of the phylogeography of hantaviruses, may provide valuable insights into the evolution of hantaviruses in the Americas.

687

PREVENTING NIPAH VIRUS TRANSMISSION: UNDERSTANDING EFFICACY OF BAMBOO SKIRT TO IMPEDE DATE PALM SAP CONTAMINATION BY BATS

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Drinking raw date palm sap is a national delicacy in Bangladesh, but is an occasional pathway for Nipah virus to move from its reservoir host in fruit bats to humans. We previously identified that fruit bats frequently visited date palm trees and contaminated the sap through their saliva and urine. We also identified that the application of bamboo skirts around the date palm sap collection pots was a promising intervention to prevent date palm sap contamination. In this study, we assessed the efficacy of bamboo skirts in preventing bat's access to date palm sap. We selected 10 tall sap producing trees out of 54 by simple randomization. We matched them with a control according to their height, shaving pattern and geographical location from the rest. On only one of the matched pairs of trees a date palm sap collector placed bamboo skirt, covering the shaved part, sap stream, tap, and the collection pot. An infrared motion sensor night vision camera was placed on each pair of trees from 5:00 PM to 6:00 AM. After observing each of the 20 trees for one night, we removed the skirts from the intervention trees and placed them on the trees that were previously controls, and observed all 20 tress for an additional night. Photographic data was used to identify if and how bats accessed the sap through a bat landing, licking or urinating on the date palm's shaved part, sap stream, tap or collection pot. The proportion of date palm sap contamination by bats was significantly lower in trees protected with bamboo skirts compared to using no bamboo skirts [35% vs. 85%, OR 10.5, 95% CI 1.9-70.8, p= 0.003]. Out of the 20 nights of observation of trees with bamboo skirts, bats were seen in and around 12 trees (60%): bats contaminated date palm sap in seven out of twelve (58%) observations. The contaminations either occurred with faulty bamboo skirt placement and/or the skirt was not wide enough to cover entire shaved part. Among the 20 skirts placed, 13 (65%) were wide enough to cover the shaved part and had been properly applied. Among these 13, bats were unable to get any access to the sap. Conclusion: Bamboo skirts wide enough to cover the entire shaved part of a date palm tree and carefully applied effectively protects date palm sap contamination by bats. We now need to identify approaches to scale up the appropriate application of properly sized bamboo skirts.

688

CHIKUNGUNYA VIRUS POPULATION DYNAMICS IN CELLS AND *AEDES ALBOPICTUS*

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Arthropod-borne RNA viruses can cause epidemics by changing their host ranges to increase infection of humans. Adaptation to the urban mosquito *Aedes albopictus* may have expanded a 2005-6 outbreak of chikungunya virus (CHIKV) in Reunion Island that subsequently circulated among humans in the absence of other amplifying hosts. However, despite frequent arbovirus emergence, viral evolutionary processes that mediate host range changes are poorly understood. Little is known about how arbovirus population dynamics influence host infection and transmission. Dengue and West Nile viruses exist as quasispecies in patients and mosquitoes and arboviruses undergo bottlenecks during mosquito infection, but no experiments have examined how quasispecies diversity influences infection dynamics and transmission by vectors. To this end, and to better understand arbovirus population structures *in vitro*, we characterized quasispecies diversity and genetic distance in variously passaged CHIKV populations. CHIKV rescued from an infectious

clone and its Reunion Island progenitor strain were serially passaged in vertebrate or mosquito cells, or alternately passaged between vertebrate and mosquito cells, to simulate natural transmission. To expand diversity, clonal CHIKV was subjected to chemical mutagenesis. CHIKV populations were characterized from vector midguts and salivary glands. Envelope gene diversity and distance were compared in CHIKV sequences from cell culture supernatants and homogenized mosquito organs to determine: 1) differences in virus populations resulting from serial vertebrate or mosquito cell passage, 2) common sequence signatures in serially and alternately passaged populations, 3) whether the ingested consensus sequence infects and is transmitted by CHIKV vectors and 4) how varying CHIKV diversity affects rates of vector infection and transmission. These arbovirus quasispecies dynamics studies will define the extent and nature of CHIKV diversity in cells and mosquitoes and will improve understanding of how arbovirus diversity influences transmission by vectors.

689

ISOLATION AND PHYLOGENETIC ANALYSIS OF MUCAMBO VIRUS (VENEZUELAN EQUINE ENCEPHALITIS COMPLEX SUBTYPE IIIA) IN TRINIDAD

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In the 1950s and 1960s, alphaviruses in the Venezuelan equine encephalitis (VEE) antigenic complex were the most frequently isolated arboviruses in Trinidad. Since then, there has been very little research performed with these viruses. Herein, we report on the isolation, sequencing, and phylogenetic analyses of Mucambo virus (MUCV; VEE complex subtype IIIA), including 6 recently isolated from *Culex (Melanoconian) portesi* mosquitoes and 11 previously isolated in Trinidad and Brazil. Results show that nucleotide and amino acid identities across the complete structural polyprotein for the MUCV isolates were 96.6 - 100% and 98.7 - 100%, respectively, and the phylogenetic tree inferred for MUCV was highly geographically- and temporally- structured. Bayesian analyses suggest the sampled MUCV lineages have a recent common ancestry of approximately 198 years (with a 95% highest posterior density (HPD) interval of 63 - 448 years) prior to 2007, and an overall rate of evolution of 1.28×10^{-4} substitutions/site/yr.

690

TRACKING *TOXOPLASMA GONDII* FROM LAND TO SEA

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With a global distribution and capacity to potentially infect all cell types in most warm blooded animals, *Toxoplasma gondii* is arguably the most successful zoonotic parasite in the world. Although the parasite's ability to undergo sexual multiplication in the feline intestine is not essential for the sustainability and expansion of the parasite population, it provides *T. gondii* with an opportunity for greater survival through genetic recombination and wider distribution in the environment with the dissemination of oocysts in the feces of its felid definitive hosts. The increasing popularity of cats as pets, and common practice of allowing these cats to deposit feces outside, as well as the maintenance of feral cat colonies provides ample opportunities for *T. gondii* oocysts to enter the environment and be transmitted to other animals, including humans and wildlife. Results will be presented from our National Science Foundation-funded Ecology of Infectious Disease research undertaken 2005-2009 to understand the ecological determinants of *T. gondii* transmission from wild and domestic terrestrial felids to the threatened southern sea otter population in coastal California. Detailed studies on sea otters and their prey have provided clues to potential sources of infection and the association of infection with dietary specialization which otters may have adapted to accommodate limited food resources. Surrogate microspheres which mimic the behavior of oocysts in aquatic habitats are also being utilized in transport studies to test the hypothesis that estuarine wetland degradation increases the transport of *T. gondii* oocysts from land to sea. The data collected in these transdisciplinary studies are being used to develop predictive models to evaluate the impact of changes in cat abundance, infection prevalence, habitat structure and prey selection, with the aim to guide management strategies to reduce the exposure of humans and sensitive wildlife species to *T. gondii*.

691

PUBLIC HEALTH SAFETY OF RECREATIONAL BEACH WATERS

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During summer months, samples of potable recreational bathing water were tested weekly for human waterborne pathogens in association with high and low bather numbers during weekends and weekdays, respectively. The numbers of bathers on weekends were significantly higher than on weekdays ($P<0.001$) and this was associated with a significant ($P<0.04$) increase in water turbidity. The proportion of water samples containing *Cryptosporidium parvum* oocysts, *Giardia duodenalis* cysts and *Enterocytozoon bieneusi* spores was significantly higher on weekends than on weekdays, and significantly ($P<0.01$) correlated in time, with *Enterococci* counts. The concentration of all pathogens was significantly correlated with bather density ($P < 0.01$). The study demonstrated that: a) human pathogens were present in beach water on days deemed acceptable for bathing according to fecal bacterial standards; b) the *Enterococci* count was a good indicator for the presence of *Cryptosporidium*, *Giardia*, and microsporidial spores in recreational beach water; c) water should be tested for *Enterococci* during times when bather numbers are high; d) resuspension of bottom sediments by bathers caused elevated levels of *Enterococci* and waterborne parasites in the water, thus bathers themselves can create a non-point source for water contamination; and e) exposure to recreational bathing waters can play a role in epidemiology of microsporidiosis. In order to protect public health it is recommended to: a) prevent diapered children from entering beach water: b) introduce bather number limits to recreational areas; c) advise people with gastroenteritis to avoid bathing; and d) use showers prior to bathing.

692

EFFECT OF CHALLENGE INFECTIONS ON THE IMMUNE RESPONSE OF MICE PREVIOUSLY INFECTED WITH *TOXOCARA CANIS*, *TOXOPLASMA GONDII* OR BOTH PARASITES

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Toxocara canis (Tc) and *Toxoplasma gondii* (Tg) are ubiquitous intestinal parasites of dogs and cats, respectively. The seroprevalence of *T. canis* and *T. gondii* in humans is estimated to be between 10% and 50%, and dual infections are common (OR=1.9). To begin to understand the host's immune response to concomitant infections, the cross-regulatory effects on concomitant Tc and Tg infections were examined in a murine

model. *Toxocara* infection is characterized by a Type 2 cytokine response dominated by the production of IL-4, IL-5, IL-6, and IL-10, while a response to *Toxoplasma* is characterized by production of Type 1 cytokines: TNF-α, IFNγ, IL-2, and IL-12. C57BL/6 and BALB/c mice, known to be strong Type 1 and Type 2 responders, respectively, were used. A 4x4 grid design was used to group mouse strain and treatment. For each strain, 20 mice were inoculated with Tc (100 eggs), 15 with Tg (100 oocysts), 10 with both (B), and 20 were uninoculated controls (0). Day 35 post-infection, groups were subdivided (5 mice each) and inoculated with the reciprocal cross, e.g., each of the four groups of Tc-mice were given either 500 Tc eggs (Tc/Tc), 100 Tg oocysts (Tc/Tg), both pathogens (Tc/B), or neither (Tc/0). The original design included three missing groups (B/Tg, B/B, and Tg/B). On day 9 after the date of the second inoculation, serum was collected. Cytokines in the sera were measured using Biosource Mouse Cytokine 10-Plex read on Luminex 100™. For C57BL/6 mice, a preexisting Tg infection markedly reduced the response of TNF-α, IFN-γ, IL-2, and IL12 to a secondary infection with Tc compared to mice initially infected with Tc. In the case of the BALB/c mice, there was no TNF-α or IFN-γ response given a second Tc infection, making comparisons difficult. C57BL/6 mice challenged with Tc mounted no IL-4, IL-6, or IL-10 response to Tc when first infected with Tg; BALB/c mice were the same with only exception being a slight elevation in IL-10. The primary infection appears to have a marked effect on the response to the secondary infection.

693

APPLICATION OF A TRNA REPEAT UNIT GENOTYPING METHOD TO CLINICAL ISOLATES OF *ENTAMOEBA HISTOLYTICA*

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Only 10% of those infected with *Entamoeba histolytica* develop symptomatic disease. The factors determining whether a person infected with *E. histolytica* develops invasive disease are poorly understood. Molecular genotyping by targeting polymorphic genetic loci may aid in the close examination of the population structure of *E. histolytica* clinical isolates. In the present study, we analyzed six tRNA-linked polymorphic short tandem repeats loci among stool isolates received at the Toronto Public Health Laboratories from seven patients who were either asymptomatic, had diarrhea/dysentery, or had developed a liver abscess. The study included a case of a hypervirulent outbreak strain in a traveler returning from Italy. The genetic relatedness among the isolates was analyzed by performing the pars parsimony analysis using Phylip 3.68 software on alphanumeric annotation data and raw nucleotide sequence data of tRNA-linked STRs. The genotyping analysis revealed six different *E. histolytica* genotypes. The most remarkable and extensive variations among the six t RNA STR loci was found in the outbreak strain. The hyopervirulent outbreak strain was genetically distinct from *E. histolytica* sensu stricto strains obtained from patients with either diarrhea or who were asymptomatic. Two isolates from asymptomatic individuals residing at the same household were epidemiologically linked. Interestingly, the outbreak strain which was responsible for highly virulent disease (ALA, elevated serology) was most genetically similar to HM1: IMSS, a reference strain originally isolated from a patient with invasive colitis. The parsimony analysis generated trees of similar topology with both alphanumeric annotation data and raw nucleotide sequence data of tRNA-linked STRs. The alphanumeric annotation analysis fits well with the present tRNA STR schema wherein the genotyping is based on the STR repeats in each block of the respective tRNA loci sequence. In conclusion, the results showed that the hypervirulent outbreak isolate was genetically distinct from isolates genotyped from patients who were asymptomatic or had diarrhea alone.. This genotyping schema may be useful to distinguish virulent strains from less virulent strains of *E. histolytica* sensu stricto. The alpha numeric annotation can be an alternative to the raw sequence data analysis in tRNA-linked STR genotyping.

694

ROLE OF NF-KB RESPONSES AND APOPTOSIS IN MAINTAINING EPITHELIAL HOMEOSTASIS DURING *ENTAMOEBA HISTOLYTICA* INFECTION

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Intestinal infection with *Entamoeba histolytica* in mice follows a strain dependent outcome, where CBA mice are relatively susceptible to persistent infection, C57BL/6 mice clear rapidly, and 129 strains are intermediate. This phenotype tracks with the strain's non-hemopoietic cells, suggesting the epithelium plays a major role, however the mechanism is not clear. In this work we examined the epithelial NF-κB response to the parasite. We found that the NF-κB subunit p50 predominated in nuclear extracts of C57BL/6 cecal tissue after *E. histolytica* challenge, while both p50 and p65 were found in CBA and 129 mice. Presence of p50 was protective, in that C57BL/6 and 129 mice with targeted deletion of this subunit were more susceptible to *E. histolytica* infection as measured by culture, cecal parasite ELISA, and/or histologic scores. Transepithelial electrical resistance of cecal explants from 129 and C57BL/6 p50 KO mice suffered in response to the parasite compared with their WT counterparts, suggesting a protective function for p50 resided in the epithelium itself. Pharmacologic inhibition of p50 nuclear translocation in C57BL/6 WT cecal explants recapitulated the TEER decline of C57BL/6 p50 KO cecal explants, suggesting the decline in p50 KO explants was not due to acquired defects in their epithelium, and that active translocation of p50 during amebic infection mediates protection. Taken together this work shows that NF-κB subunit translocation in epithelial cells is an actively regulated and strain-variable process that can protect the intestine from *E. histolytica* challenge.

695

HIGH DOSE OF VITAMIN A SUPPLEMENTATION PROTECTS ABORIGINAL SCHOOLCHILDREN IN RURAL MALAYSIA FROM *GIARDIA DUODENALIS* REINFECTION: A RANDOMIZED CONTROLLED TRIAL

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Giardiasis, caused by *Giardia duodenalis*, is considered the most common protozoal infection in humans particularly among children in developing countries. A randomized, double-blind, placebo-controlled trial was carried out on 250 aboriginal schoolchildren in Pos Betau, Pahang, Malaysia to investigate the effects of a mega dose of vitamin A supplementation (200 000 IU) on *G. duodenalis* reinfection. Faecal samples were examined for the presence of trophozoites and/or cysts of *Giardia* by trichrome staining technique before and after 14 days of receiving a 3-days course of 400 mg/daily of albendazole tablets. The effect of the supplements was assessed at 3 and 6 months after the receiving of interventions (vitamin A or its identical placebo). The overall prevalence of giardiasis was 17.8%. There was no significant difference in the prevalence of giardiasis between males and females (χ^2=1.37; *P*=0.242) while children aged >10 years had a significantly higher prevalence than those aged ≤10 years (χ^2=3.91; *P*=0.048). The findings showed that the reinfection rate of *Giardia* was high at 6 months assessment and the reinfection rate was significantly lower among vitamin A supplemented-children compared to those in placebo group (*P*=0.018). In conclusion, vitamin A supplementation showed a positive impact in reducing *Giardia* reinfection rate and the effect could be immune-mediated as the reported role of vitamin A in the production of IgA which has anti-*Giardia* properties. Albendazole tablets and vitamin A supplementation should be distributed periodically to these children in order to reduce the prevalence of giardiasis significantly and to improve their health and nutritional status.