therapy is recommended in the civilian sector, a 2-day regimen of DP is currently used by the Cambodian military to improve compliance. As part of an active observational malaria epidemiology cohort study in Oddar Meancheay Province, an area of high transmission in northern Cambodia, 200 healthy volunteers were enrolled and followed weekly for up to 4 months. All subjects developing uncomplicated malaria were randomized to receive directly observed therapy with 320/2880 mg of DHA-piperaquine given over 2 or 3 days (n = 40 per arm). Subjects were followed weekly for a minimum of 42 days and assessed for treatment efficacy, safety and tolerability. The trial was powered (80%) to detect an expected 25% higher recurrence rate in the 2-day group compared to 3 days. From September 2010 to February 2011, 80 malaria patients were randomized to DP, 16 (20%) with *P. falciparum*, 61 (76%) with *P. vivax* and 3 (4%) with mixed infection. PCR-uncorrected per protocol 42-day efficacy rates against all malaria species combined were not statistically significantly different between treatment groups: 89% (95% CI 76-96%) for 2 days and 92% (95% CI 80-97%) for 3 days. Intention to treat efficacy rates were also not significantly different: 83% (95% CI 68-91%) for 2 days and 88% (95% CI 74-95%) for 3 days. Median parasite clearance times were 11.1 hours for *Plasmodium vivax*, but 72.5 hours for *P. falciparum*; there were no significant differences between treatment groups. DP was safe and well tolerated without significant treatment-related adverse events. PCR uncorrected all-malaria efficacy was not significantly different between 2 and 3 days of DP in this population on the Thai-Cambodian border. However, 42-day cure rates appear to be lower than previously reported. Given the proximity of this study site to areas of known multi-drug resistance this finding is concerning.

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CHROMOSOMAL INTEGRATION OF TRANSGENES AND DERIVATION OF A STABLE TRANSGENIC LINE IN THE PARASITIC NEMATODE *STRONGYLOIDES RATTI*

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Parasitic nematode infections adversely affect over one billion people. Genetic transformation is a potential tool for analyzing gene function to identify new drug and vaccine targets in these worms. We have developed a robust system for transgenesis in *Strongyloides* spp. using gonadal microinjection for gene transfer. Using this system, transgenes are expressed in promoter-regulated fashion in the F1 but are silenced in subsequent generations, presumably because of their location in repetitive episomal arrays. To counteract this silencing, we explored transposon-mediated chromosomal integration of transgenes in *S. ratti*. To this end, we constructed a donor vector encoding green fluorescent protein (GFP) under the control of the *Ss-act*-2 promoter with flanking inverted tandem repeats specific for the *piggyBac* transposon. Free-living *Strongyloides ratti* females were transformed with this donor vector and a helper plasmid encoding the *piggyBac* transposase. The transgene was detected in the F1 and later generations by PCR, and 15.8% of F1 larvae were GFP-positive. We inoculated a rat with 34 F1 GFP-positive infective larvae (L3i), and 0.48% of 6014 F2 individuals resulting from this host passage expressed GFP. We cultured GFP-positive F2 individuals to produce GFP-positive F3 L3i for additional rounds of host and culture passage. GFP expression frequencies in subsequent generations were 74.24% in F3, 98.99% in F4, 82.39% in F5 and 100% in F6. The resulting transgenic line now has uniform GFP expression among all progeny. Chromosomal integration of the reporter transgene in *S. ratti* was confirmed by Splinkerette PCR, which revealed the transgene flanked by *S. ratti* genomic sequences corresponding to at least three discrete integration sites. BLAST searches of flanking sequences against the *S. ratti* genome revealed integrations in three contigs: 75336 (position 3211), 74996 (position 155901) and 74278 (position 172601). This result provides the basis for two powerful functional genomic tools in *S. ratti*: hertiable transgenesis and insertional mutagenesis.

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NEOMYCIN SELECTION OF TRANSGENIC *SCHISTOSOMA MANSONI*

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Draft genome sequences for *Schistosoma mansoni* and *S. japonicum* were reported recently, a landmark event that ushered in the post-genomic era for schistosomiasis. Analysis of target genes to underpin new interventions for schistosomiasis requires functional genomics approaches such transgenesis that will validate essential genes to be targeted with drugs or vaccines. We have adapted murine leukemia retrovirus (MLV) vectors -widely used in human gene therapy research- to transduce schistosomes, leading to integration of reporter transgenes into the parasite genome. Drug selection of transgenic schistosomes would be highly desirable in order to provide a means to enrich for populations of transgenic worms in virion-exposed parasites. Given that *neoR* (the gene encoding resistance to neomycin/G418) driven by the MLV's 5'-LTR as promoter is actively expressed in schistosome tissues, and that G418 is lethal under the conditions tested here, we investigated whether MLV transduced schistosomules could be rescued on G418. First, a dose-response kill curve and lethal G418 concentrations were established. Second, one day old schistosomules were infected with MLV at two concentrations of virions, 1X and 3X. Transduced worms were cultured with or without G418 and by day 10, aliquots of schistosomules from the groups were stained for viability with Trypan blue and enumerated. No significant differences were observed among the group of parasites without G418. However, significant differences were found among schistosomules cultured with G418 where more schistosomes survived when transduced with virions (3x) in comparison to controls (p=0.0039). Remarkably, *neoR* expression levels in the group subjected to G418 selection was higher than in worms treated with the same titer of virus but cultured without G418. This likely reflects enrichment of transgenic schistosomules within the population of transduced parasites subjected to G418 pressure. This appears to be the first report of antibiotic selection of transgenic schistosomes or indeed of any transgenic helminth parasite species.

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THE COMPLETE *WOLBACHIA* GENOME AND TRANSCRIPTOME FROM *ONCHOCERCA OCHENGI* INDICATES A DIFFERENT WORM-SYMBIONT RELATIONSHIP TO THAT OF *BRUGIA MALAYI*

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The filarial nematode *Onchocerca ochengi*, a parasite of cattle, is recognised as the closest relative of *O. volvulus*, the aetiological agent of human onchocerciasis. In common with the filariae that cause lymphatic filariasis (including *Brugia malayi*), *O. ochengi* and *O. volvulus* contain *Wolbachia* endobacteria in the hypodermal cords of both sexes and in the reproductive tract of female worms. *Wolbachia*, which are much more prevalent in arthropods than in nematodes, are divided into approximately 10 supergroups. Four complete *Wolbachia* genomes have been published to date: two in supergroup A (from *Drosophila* spp. hosts), one in supergroup B (from a mosquito host), and one in supergroup D (strain *w*Bm from *B. malayi*). Here, we report the first complete genome of a supergroup C *Wolbachia* (strain *w*Oo from *O. ochengi*), alongside complete endobacterial transcriptomes obtained by deep sequencing of cDNA from both hypodermal cord and female reproductive tract tissues. At 0.96 Mb, the *w*Oo genome is the smallest thus far characterised for any *Wolbachia* and is 11% smaller than that of *w*Bm. In contrast to *w*Bm,

the *w*Oo genome contains very few insertion sequences and fewer intact ankyrin-repeat containing genes. Key metabolic pathways for heme and riboflavin, which have been hypothesised to form the basis of a nutritional mutualism between *w*Bm and its worm host, show evidence of pseudogenization in *w*Oo and transcription across both pathways is low, irrespective of anatomical location in the worm. The *w*Oo transcriptome is dominated by chaperonin and chaperone proteins, translation machinery and enzymes involved in nucleotide metabolism. Approximately 100 proteins encoded by abundant *w*Oo transcripts were detected in worm homogenates by proteomic methods. Three of these are uncharacterised membrane proteins, two of which do not have orthologues in *w*Bm. Taken together, these analyses indicate important dissimilarities between the genomes of *w*Bm and *w*Oo, and suggest that immuno-defensive (as opposed to nutritional) mutualism may be the major phenotypic role of *w*Oo in its worm host.

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FUNCTIONAL GENOMICS APPROACHES TO STUDYING *SCHISTOSOMA MANSONI* FEMALE WORM DEVELOPMENT

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Schistosomiasis affects more than 200 million people worldwide making it a major cause of morbidity and mortality globally. Although the genome sequence for *Schistosoma mansoni* has been determined, few functional genomics techniques have been developed for use in *S. mansoni,* limiting the usefulness of the sequence data. Using serial analysis of gene expression (SAGE) Williams et al (2007) elucidated transcriptional differences between life cycle phases. Using the SAGE data we have identified transcripts that are preferentially expressed in reproductively mature females. Using bioinformatics, we have linked these SAGE tags to unique protein sequences; however, many of these female-specific proteins do not have a predicted function. To functionally characterize these transcripts we have developed a whole mount *in situ* hybridization (WISH) method that can be used to identify the tissue-specific expression of transcripts in intact, adult *S. mansoni* worms (Cogswell et al 2011). To validate these protocols we determined the tissue-specific expression of *tetraspanin 2, phenol oxidase*, the secretory *Cu/Zn superoxide dismutase*, and an Argonaute family member. The localization of these transcripts by WISH correlates with prior studies performed using other methods, indicating that WISH will be a useful functional genomics tool. We have also identified a variety of cell-specific markers (Collins et al, 2011) to be used in conjunction with WISH to pinpoint the tissue-specificity of expression of female-specific transcripts providing more information about their function. Using WISH we have localized expression of four female-enriched transcripts specifically to the vitellaria/vitelline duct and three transcripts to the ovary, oviduct, and/or ootype. In order to determine the function of these female-enriched transcripts we have investigated the use of the phylogenetically related flatworm *Schmidtea mediterranea* as a model for schistosome biology. We have identified orthologs of many of the *S. mansoni* female-enriched genes in *S. mediterranea.* We have localized several of these orthologs in *S. mediterranea* using WISH and find that they localize to reproductive tissues in the same way that we observed in *S. mansoni.* Currently we are using established RNA interference techniques to silence orthologs in both worm species to examine what role they play in female development and reproductive biology.

1003

ANALYSIS OF TRANSCRIPTIONAL REGULATION OF TETRACYCLINE RESPONSIVE GENES IN *BRUGIA MALAYI*

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Most filarial parasites, including *Brugia malayi* harbor an endosymbiotic bacterium of the genus *Wolbachia*. While the physiological role of the endosymbiont remains unclear, it's elimination by doxycycline or tetracycline treatment results in sterilization of the adult parasite. Previous studies have demonstrated that the mRNA pools of certain nuclearly encoded genes are increased in parasites exposed to tetracycline, implicating these as potentially important players in the *B. malayi - Wolbachia* interaction process. It is possible to hypothesize that the increase in the stable mRNA levels of these genes results from up-regulation of transcription and that this involves cis acting regulatory sequences present in the gene's promoters. To test this hypothesis, the responsiveness of three promoters derived from genes whose mRNAs were increased by tetracycline treatment were tested in a homologous *B. malayi* transfection system. Reporter gene activity driven from all three promoters was found to increase upon exposure to tetracycline, consistent suggesting that transcription was up-regulated in response to tetracycline. The element responsible for tetracycline responsiveness was mapped in one of these promoters, BmHSP70. Mutation of the stress response element in the BmHSP70 promoter did not result in any change in tetracycline responsiveness. However, mutation of a TATAA box-like motif present in the promoter resulted in loss of the tetracycline response. These studies provide evidence supporting the hypothesis that changes in mRNA levels in response to tetracycline treatment are regulated at the transcriptional level and that this is accomplished through a novel use of an element normally employed as part of the core of many eucaryotic promoters.

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EVIDENCE OF EFFICIENT TRANSOVARIAL TRANSMISSION OF CULEX FLAVIVIRUS BY *CULEX PIPIENS* (*DIPTERA: CULICIDAE*)

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The purpose of this study was to determine the transovarial transmission (TOT) potential and tissue tropisms of Culex flavivirus (CxFV), an insect-specific flavivirus, in *Culex pipiens* (Linnaeus). Several hundred mosquito egg rafts were collected in the field, transferred to the insectaries, reared to the fourth larval stage and identified using morphological characteristics. *Cx. pipiens* were reared to adults, allowed to oviposit in individual containers and tested for CxFV RNA by reverse transcription-polymerase chain reaction (RT-PCR) and nucleotide sequencing. Eighteen CxFV RNA-positive females were identified. Thirty F_1 adults from each positive female were individually tested by RT-PCR for CxFV RNA. Viral RNA was detected in 526 of 540 progeny and thus, the filial infection rate was 97.4%. Because all 18 females produced infected offspring, the TOT rate was 100%. These data suggest that efficient TOT of CxFV occurs in nature. To define the tissue tropisms of CxFV, different tissues (salivary glands, ovaries, testes, head, fat bodies and midguts) were removed from the remainder of the F_1 and tested by RT-PCR for CxFV RNA. Viral RNA was detected in all tissues. Additionally, uninfected laboratory-colonized *Cx. pipiens* were infected with CxFV by needle inoculation, and ovaries were collected at 4, 6, 8 and 12 days post-inoculation and tested for CxFV RNA by RT-PCR. Viral RNA was detected at all time points demonstrating that

CxFV reaches the ovaries as early as 4 days post-inoculation. Surprisingly, however, we were unable to demonstrate transovarial transmission despite the presence of viral RNA in the ovaries. Nevertheless, the experiments performed with field-infected *Cx. pipiens* demonstrate that TOT is an efficient mechanism by which CxFV is maintained in mosquitoes in nature.

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CHIKUNGUNYA VIRUS IN VERTEBRATES

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus (family *Togaviridae)* endemic to Africa and much of Southeast Asia that can cause severe debilitating fever and arthralgia in humans. The African strains of CHIKV are thought to have a sylvatic cycle maintained by rodents and forest mosquitoes with occasional outbreaks in human populations. In Asia, there is no known sylvatic reservoir and the virus is proposed to circulate exclusively in humans. Recent viral evolutionary changes have been linked to increased virulence and altered vector specificity, such that some recent outbreaks have been associated primarily with *Ae albopictus,* while historically *Ae aegypti* have been the predominant human vector. The purpose of this study was to investigate the potential of various domestic and wild vertebrates to become infected with and possibly contribute to the spread of CHIKV. We tested more than 30 species representing all four classes of terrestrial vertebrates: reptiles, amphibians, mammals and birds. Animals were inoculated subcutaneously with 10,000-100,000 plaque-forming units of either a South African strain of CHIKV or an isolate from the Comoros Islands 2005 outbreak. Blood samples were collected daily for at least six days to characterize viremia. None of the birds developed detectable viremia, and of the mammals tested, only lab rodents demonstrated viremia. However, several of the ectothermic species, including leopard frogs, garter snakes, Burmese pythons, and ball pythons became viremic and maintained virus at sufficiently high titers to potentially re-infect mosquitoes. No mortality or obvious signs of clinical illness were observed in any of these species. These experiments suggest that reptiles and amphibians may play a role in virus maintenance and spread during epidemic seasons. Additionally, it is possible that hibernating ectotherms could provide a mechanism for virus overwintering in climates where winter months don't support vector populations.

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CHIKUNGUNYA VIRUS EMERGENCE IS CONSTRAINED IN ASIA BY LINEAGE-SPECIFIC ADAPTIVE LANDSCAPES

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Adaptation of RNA viruses to a new host or vector species often results in emergence of new viral lineages. However, lineage-specific restrictions on the adaptive processes remain largely unexplored. Recently, a chikungunya virus (CHIKV) lineage of African origin emerged to cause major epidemics of severe, persistent, debilitating arthralgia in Africa and Asia. Surprisingly, this new lineage is actively replacing endemic strains in Southeast Asia that have been circulating there for 60 years. This replacement process is associated with adaptation of the invasive CHIKV strains to an atypical vector, the *Aedes albopictus* mosquito that is ubiquitously distributed in the region. Here we demonstrate that linage-specific epistatic interactions between substitutions at amino acid positions 226 and 98 of the E1 envelope glycoprotein, the latter of which likely resulted from a founder effect, have for 60 years restricted the ability of endemic Asian CHIKV strains to adapt to this new vector. This adaptive constraint appears to be allowing invasion of the unoccupied vector niche by *Ae. albopictus*-adapted African strains. These results underscore how different adaptive landscapes occupied by closely related viral genotypes can profoundly affect the outcome of viral evolution and disease emergence.

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IDENTIFICATION AND CHARACTERIZATION OF CHIKUNGUNYA VIRUS VARIANTS WITH INCREASED TRANSMISSION *IN VIVO*

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Chikungunya virus (CHIKV), an Alphavirus and member of the Togaviridae family, is a re-emerging and significant human pathogen. Like all other arboviruses, CHIKV is able to infect and face numerous selective pressures and bottlenecks in both vertebrate and invertebrates hosts, yet the molecular mechanisms involved in initiating, establishing, and maintaining these distinct infections are poorly understood. Genetic diversity and adaptive mutations have been shown essential for the evolution of CHIKV host tropism, suggesting this as a possible mechanism important in mediating viral infection. Thus, we hypothesized that genetic adaptations *in vivo* may play important roles in CHIKV transmission and pathogenesis as well. To address this hypothesis, we infected *Aedes aegypti*i mosquitoes with wildtype CHIKV and harvested infectious virus from insect midguts, legs, salivary glands, and saliva at various time points. We found evidence for genetic bottlenecks at the level of midgut and salivary glands that were overcome downstream of these events, and we subsequently sequenced viral genomes present in each fraction to determine the genetic changes that occurred over the course of infection. Interestingly, we identified a major sub-population of viruses containing two previously undescribed mutations in the E1 glycoprotein present only in the saliva samples. These mutations were individually introduced into the Chikungunya virus infectious clone, virus was produced, and both mosquitoes and mice were infected. We found both mutations to significantly increase transmission rates compared to the wildtype control, suggesting that these variants comprise a temporally or anatomically restricted sub-population important in completing the transmission cycle. Further *in vivo* and *in vitro* studies are in progress to identify the molecular mechanisms involved. These studies will not only increase our understanding of CHIKV biology but should also provide valuable insight into the mechanisms of arbovirus evolution and pathogenesis.

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PRECLINICAL DEVELOPMENT OF A LIVE-ATTENUATED CHIKUNGUNYA VACCINE

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Recently, Chikungunya virus (CHIKV), a mosquito-borne alphavirus, re-emerged in Africa and spread to islands in the Indian Ocean, the Indian subcontinent, SE Asia and Italy. Viremic travelers have also imported CHIKV to the Western hemisphere, which highlights the risk of CHIKV in naïve populations. In addition to the huge burden of arthralgic disease, which can persist for months or years, epidemiologic studies estimated case-fatality rates of ~0.1%, principally from neurologic disease in older patients. There are no licensed CHIK vaccines or effective therapies. CHIKV is endemic in many resource-poor countries, so an effective vaccine must be inexpensive to manufacture and induce rapid, long-lived immunity. To develop a live-attenuated CHIK vaccine (CHIKV-IRES), we inactivated the subgenomic promoter of CHIKV La Reunion and inserted a picornavirus internal ribosome entry site (IRES) that functions poorly in insect cells. This vaccine is highly attenuated yet immunogenic in mouse models, and

is incapable of replicating in mosquito cells. We have characterized the safety and toxicology of this vaccine strain in A129 and C57/Bl6 mouse models, and have demonstrated its attenuation as compared to the 181-/clone 25 CHIK vaccine. To support our preclinical efforts, we have characterized the genetic stability of CHIKV-IRES *in vitro*, developed quality control and release assays, and initiated GMP vaccine manufacture which will support the testing necessary to submit an IND application to begin clinical trials in humans. This innovative collaboration between academic and industrial partners will have immediate, dramatic impacts on human health in Asia and Africa where CHIKV causes both severe health effects and economic hardship. A safe and effective CHIKV vaccine could, also greatly reduce the risk of CHIKV importation and endemic establishment in the Western Hemisphere during epidemics in Africa or Asia.

1009

SINGLE DOSE RVF-VRP IMMUNIZATION CONFERS COMPLETE PROTECTION FROM LETHAL RIFT VALLEY FEVER VIRUS INFECTION REVEALING THE IMPORTANCE OF VIRAL REPLICATION FOR ANTIVIRAL IMMUNITY

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Rift Valley fever virus (RVFV) is a mosquito-borne pathogen that poses a significant threat to human and livestock health throughout Africa and the Arabian Peninsula. Abortion storms in sheep and cattle are characteristic of RVF epizootics, and mortality of young animals can approach 100%. RVFV infection in humans is generally associated with a self-limiting febrile illness, but in a small percentage of cases (1-2%), it can progress to severe hepatitis, delayed-onset encephalitis, retinitis or a hemorrhagic syndrome with case fatality of 10 to 20%. In an effort to improve upon previously reported virus-like particle (VLP) vaccine constructs, we developed a system for producing high-titered (>$5.0x10^6$ FFU/ml) infectious non-spreading viral replicon particles (RVF-VRP). These constructs differ from authentic RVF virus in that RVF-VRP undergo only one round of infection; new particles cannot be produced in infected cells due to the absence of the glycoprotein-encoding viral M segment. Unlike standard VLP, RVF-VRP contain the full-length viral RdRp-encoding L segment and the nucleoprotein-encoding S segment, allowing *de novo* synthesis of all viral and/or marker proteins (i.e., GFP and luciferase). This active replication within infected cells suggests RVF-VRP should be more highly immunogenic than traditional VLP vaccine constructs. A single dose immunization with RVF-VRP ($1x10^5$ FFU SC) in C57BL/6 mice provided 100% protection from lethal RVFV challenge ($1x10^5$ PFU SC) at 28 days post-immunization. In contrast, immunization with non-replicating VLP controls resulted in lower survivorship after lethal virus challenge, indicating RdRp-mediated replication is important for the development of an effective immune response. Future studies will elucidate the mechanism by which viral replication in host cells enhances the immune response to protect against this significant health threat.

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HUMAN MONKEYPOX IN THE AFTERMATH OF SMALLPOX ERADICATION AND THE RISK OF SUSTAINED HUMAN-TO-HUMAN TRANSMISSION

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Since the eradication of smallpox three decades ago, human cases of zoonotic orthopoxvirus infections have risen worldwide. The lack of continued smallpox vaccination is likely a contributing factor because it decreases population immunity, but the 20-fold increased incidence of human monkeypox in the Democratic Republic of the Congo is particularly striking. By combining contact tracing data with scar surveys indicative of existing immunity to orthopoxvirus, we show that monkeypox virus in its present form is unlikely to establish persistent circulation in the rural populations currently affected. However, viral adaptation or introduction to crowded urban settings poses major risks. Focusing on the contribution of human to human transmission against a background of zoonotic spillover, we analyze surveillance data from 1980-84 and 2005-07 to show that the marked increase in human cases cannot be explained by reduced population immunity alone, and that increased spillover probably has contributed substantially. Further, we demonstrate the importance of quantifying surveillance errors including imperfect case detection, multiplicity of primary infections and false positive cases when estimating the effective reproductive number. Thus our analysis provides perspective for future surveillance and subsequent analysis of monkeypox and other emerging zoonoses. More generally, the rise of monkeypox serves as a warning that pathogen eradication can lead to unintended consequences with potential to partially offset public health gains.

1011

DEVELOPMENT OF A RECOMBINANT PROTEIN BASED CHEMICAL CONJUGATE VACCINE TO INTERRUPT MALARIA TRANSMISSION

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The goal of elimination and eradication of *Plasmodium falciparum* (*Pf*) will only be achieved with the development of an effective malaria vaccine. Vaccines to Interrupt Malaria Transmission (VIMT) are a class of malaria vaccines recently identified as a key element to achieve this goal. A VIMT may target both pre-erythrocytic and sexual stage parasites to produce a bifunctional vaccine. To this end, a vaccine is being developed that uses recombinantly produced well characterized, non-tagged near full-length Pf circumsporozoite protein and the 25 kDa sexual stage specific protein, Pfs25M, both independently conjugated to the vaccine carrier EPA (ExoProtein A, a non-toxic mutant of ExoToxin A from *Pseudomonas aeruginosa*). Pfs25H-EPA conjugates, manufactured under cGMP, induce a significant increase in antibody responses, over unconjugated antigen, which corresponds with enhanced transmission blocking activity. Conjugation of recombinant CSP also significantly enhances CS specific antibody responses and may broaden the breadth of the response against the amino- and carboxyl-terminal ends. Identification of a common platform for adjuvant formulation is being initiated. Additional parasite proteins are under pre-clinical development, including the scalable production of another transmission blocking vaccine candidate Pfs230, which induces optimum transmission blocking activity in the presence of human complement. Altogether, this platform enhances the potential to develop an effective bifunctional VIMT vaccine.

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IMMUNOGENICITY OF MIXED AND SINGLE ALLELE VACCINES OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN REGION II

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The Duffy Binding protein of *Plasmodium vivax* is essential for host erythrocyte invasion. DBP region II (DBPII) contains critical residues for receptor recognition thereby making the molecule an attractive vaccine candidate against *P. vivax* blood stages. Immune responses to DBP have been shown to inhibit erythrocyte binding and invasion. Similar to other blood-stage antigens, allelic variation within the DBPII and associated strain specific immunity may be a major challenge for development of a broadly effective vaccine against *vivax* malaria. We hypothesized that immunization with a multiple allele vaccine will be more effective in producing a broadly reactive and inhibitory antibody response to diverse DBPII alleles than a single allele vaccine. In this study, we compared single PvDBPII allele immunizations (Sal1, 7.18, P) with a combination of the same alleles. Quantitative analysis by ELISA demonstrated that the immunogenicity of the multiple allele vaccine strategy generally performed better when tested for reactivity against heterologous variant DBPII alleles, with significantly higher antibody titers induced by some of the single allele immunizations. Qualitative analysis by *in vitro* erythrocyte-binding inhibition assays demonstrated that the multiple allele immunization strategy overall produces a broader binding-inhibitory antibody response, even to alleles not included in the vaccine. In either case, there was no correlation between antibody titer and functional inhibition. These data suggest that a multiple allele vaccine may enhance immunogenicity of a PvDBPII vaccine and requires further investigation to optimize.

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EVALUATION OF THE *PLASMODIUM FALCIPARUM* ERYTHROCYTE INVASION LIGAND PFRH4 AS A TARGET OF PROTECTIVE IMMUNITY AND VACCINE CANDIDATE

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Repeated exposure to *Plasmodium falciparum* in humans eventually results in protective immunity that prevents symptomatic malaria and high-density parasitemia. Antibodies to *P. falciparum* merozoite antigens are thought to play an important role, but their targets have been poorly defined, few merozoite antigens have been examined, and antibody effector mechanisms are not well understood. Additionally, there is limited knowledge to guide the selection of candidate antigens for vaccine development. The *P. falciparum* reticulocyte binding homologues (PfRh1, PfRh2, PfRh4, and PfRh5) play an important role in invasion of erythrocytes and are potential vaccine candidates. PfRh4 plays a key role in sialic acid-independent invasion of erythrocytes and binds to CR1. However, the importance of PfRh4 as a target of acquired immunity has not been established and its expression is known to vary between different isolates. In this study, we undertook a comprehensive analysis of the significance of PfRh4 as a target of human immunity and its potential as a vaccine candidate. Using recombinant PfRh4 proteins we assessed the acquisition of antibodies to PfRh4 among a longitudinal cohort of 200 children in Papua New Guinea. Total IgG and IgG subclasses were assessed and prospectively related to the risk of symptomatic malaria and parasitemia of different densities. Antibodies against the erythrocyte-binding region of PfRh4 were affinity-purified from human sera and were shown to be potent inhibitors of erythrocyte invasion. Sequence analysis of PfRh4 identified no significant polymorphism and thus no evidence for diversifying selection. Examining PfRh4 expression in clinical *P. falciparum* isolates derived from the cohort showed that PfRh4 was expressed among most isolates, indicating its relevance as an immune target. Our results provide important insight into the development of naturally acquired immunity and support a PfRh4 as a potential vaccine candidate.

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VACCINE DELIVERY PLATFORM IMPACTS INHIBITORY ANTIBODY CROSS-REACTIVITY OF $MSP1_{42}$-BASED VACCINE

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Plasmodium falciparum MSP1 is a leading erythrocytic-stage malaria vaccine candidate. The 195kDa protein is processed into several fragments, and has been implicated in the initial binding of erythrocytes by merozoites. Prior to erythrocyte invasion, the C-terminal fragment known as $MSP1_{42}$ undergoes secondary processing yielding a 33 and a 19kDa fragment ($MSP1_{19}$). Although results from seroepidemiological studies have suggested that antibodies to fragments of $MSP1_{42}$, particularly $MSP1_{19}$, may correlate with reduced clinical disease and/or parasite densities, no $MSP1_{42}$-based vaccine to date has yielded clinical efficacy.

Development of efficacious blood-stage vaccines is complicated by pre-existing immunity and heterogeneity of circulating parasites observed in the field, thus we have undertaken to evaluate several approaches for delivering $MSP1_{42}$-based vaccines in rabbits. Our previous studies have shown that immunizing rabbits with recombinant $MSP1_{42}$ using a potent adjuvant, Complete Freund's, resulted in high antibody titers that cross-react against heterologous strains. We evaluated two approaches for delivering $MSP1_{42}$; one expressing the antigen on the surface or in the periplasmic space of inactivated *Escherichia coli* designated GeMI-Vax, and a second, admixture of the antigen with Neisseria Outer Membrane vesicles (NOMs) in order to develop a more clinically suitable vaccine. Both of these platforms are self-adjuvanted and thus do not require additional immunostimulatory components. $MSP1_{42}$specific antibodies will be assessed for their ability to inhibit parasite invasion and growth using a pLDH GIA against homologous and heterologous parasite strains. In addition, antibody fine specificities will be evaluated using allele specific $MSP1_{42}$ ELISAs and fragment-specific particle-based Luminex. Results from these studies will guide the development of second generation $MSP1_{42}$-based malaria vaccines and may transcend the issues of allele-specific monovalent subunit vaccines.

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POTENT VACCINE PLATFORMS CAN PROVOKE CIRCUMSPOROZOITE (CS) PROTEIN MEDIATED IMMUNOSUPPRESSION, A PARADOX OVERCOME BY CS VACCINES EXPRESSING EAT-2

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Malaria greatly impacts the health and wellbeing of over half of the world's population. Promising malaria vaccine candidates have attempted to induce adaptive immune responses to Circumsporozoite (CS) protein. Despite the inclusion of potent adjuvants, these vaccines have limited protective efficacy. Conventional recombinant adenovirus (rAd) based vaccines expressing CS protein can also induce CS protein specific immune responses, but these are essentially equivalent to those generated after use of the CS protein subunit based vaccines. In this study we combined the use of rAds expressing CS protein along with rAds expressing novel innate immune response modulating proteins in an attempt to significantly improve the induction of CS protein specific cell mediated immune responses. BALB/cJ mice were co-vaccinated with rAd vectors expressing CS protein simultaneous with a rAd expressing either a TLR agonist (rEA) or the SLAM receptors adaptor protein (EAT-2). Paradoxically, expression of the TLR agonist uncovered a potent immunosuppressive activity inherent to expression of the CS protein, an activity that prevented the rAd vaccine from inducing CS specific adaptive immunity. Fortunately, use of the rAd vaccine expressing EAT-2 circumvented CS protein's immunosuppressive activity, and generated a fivefold increase in the number of CS protein responsive IFNγ secreting splenocytes, as well as increased the breadth of T cells responsive to peptides present in the CS protein. These improvements were positively correlated with the induction of a fourfold improvement in CS protein specific CTL functional activity *in vivo*. Our results emphasize the need for caution when incorporating CS protein into malaria vaccine platforms expressing or containing other immunostimulatory compounds, as the immunological outcomes may be unanticipated and/or counter-productive. However, expressing the SLAM receptors derived signaling adaptor EAT-2 at the same time of vaccination with CS protein can overcome these concerns, as well as significantly improve the induction of malaria antigen specific adaptive immune responses *in vivo*.

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BLOOD STAGE MEROZOITE SURFACE PROTEIN CONJUGATED TO NANOPARTICLES INDUCE POTENT PARASITE INHIBITORY ANTIBODIES

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We report the results of our proof-of-concept studies on the use of <15nm, water soluble, inorganic nanoparticles as a vaccine delivery system for a blood stage malaria vaccine. Accordingly, the recombinant malarial antigen, Merozoite Surface Protein 1 (rMSP1) of *Plasmodium falciparum* was used as the model vaccine. The rMSP1 was covalently conjugated to polymer coated quantum dot CdSe/ZnS nanoparticles (QDs) via surface carboxyl groups, forming rMSP1-QDs. Anti-MSP1 antibody responses induced by rMSP1-QDs were at least two orders of magnitude higher than those obtained with rMSP1 administered with the conventional adjuvants, Montanide ISA51 and CFA. Moreover, the immune responsiveness and the induction of parasite inhibitory antibodies were significantly more superior in mice injected with rMSP1-QDs. The rMSP1-QDs delivered via intra-peritoneal (i.p.), intra-muscular (i.m.), and subcutaneous (s.c.) routes were equally efficacious. The high level of immunogenicity using the rMSP1-QDs were achieved without further addition of other adjuvant components. Bone marrow derived dendritic cells were shown to efficiently uptake the nanoparticles which lead to their activation and expression/secretion of key cytokines. Suggesting that this may be a mode of action for the enhanced immunogenicity. This study provides promising results for the use of water soluble, inorganic nanoparticles (<15nm) as potent vehicles/platforms to enhance the immunogenicity of polypeptide antigens in adjuvant-free immunizations.

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NEXT-GENERATION POPULATION GENOMIC SEQUENCING TO IDENTIFY FUNCTIONAL MICROSATELLITE VARIATION IN *PLASMODIUM FALCIPARUM*

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Genome-wide variation studies in *Plasmodium falciparum* to date have been primarily focused on SNPs and large structural variations. Low complexity features like microsatellites are abundant in the *P. falciparum* genome (~488,000 loci); however, microsatellites have length-variation mutation rates that are orders of magnitude higher than the base substitution rate that creates SNPs. These two observations therefore suggest that microsatellites have the potential to be a significant source of functional coding and regulatory variation throughout the genome. Many studies have focused on microsatellite variation in targeted regions of the genome, but genome-wide analyses using short-read next generation sequencing data have been hindered by the high AT content (~80%) of the genome. We developed a tool to interrogate genome-wide variation in microsatellite regions of the *P. falciparum* genome using short read Illumina sequencing data, and validated its accuracy with a comparative analysis of Sanger generated sequence from the 3D7 and Dd2 strains. Polymorphic microsatellite calls based on Illumina data were validated through PCR amplification and Sanger sequencing of a subset of loci. Genotypes for ~75,000 microsatellite loci were generated from Illumina sequencing data for a sample of 25 Senegalese parasite isolates. A total of 17,940 of these loci were identified as polymorphic in at least one isolate, 8,556 of which were polymorphic in at least two isolates. Furthermore, 282 polymorphisms observed in at least two isolates fall within genic coding sequences and likely induce frame-shift mutations. Genes subject

to microsatellite-based frame-shifts are found throughout the *P. falciparum* genome and span a range of functions, including chromatin regulation and cellular metabolism. The impact of microsatellite polymorphisms on cis-regulation of gene expression is more difficult to assess, but is potentially significant given that 1648 genes have a polymorphic microsatellite within 500 bp upstream of their translational start site. The genome-wide prevalence and variability of microsatellites at the population level suggests they should be considered on par with SNPs as contributors to important functional polymorphism in the parasite.

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POPULATION GENOMIC SCAN FOR SIGNATURES OF BALANCING SELECTION AND NOVEL CANDIDATE TARGETS OF IMMUNITY IN *PLASMODIUM FALCIPARUM*

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The memory component of acquired immune responses causes frequency-dependent selection on pathogens, leading to distinctive patterns of polymorphism in genes encoding important target antigens. These are detectable by evaluating statistical signatures of balancing selection, using either frequency-based or polymorphism-versus-divergence indices. This has been well illustrated in analyses of malaria parasite antigens that are known candidate targets of naturally acquired immunity and more recently in new panels of genes expressed in merozoites. For a comprehensive screen of such signatures and to prospect for more targets of immunity among poorly known *Plasmodium falciparum* proteins, we purified parasites from >100 isolates in an endemic Gambian population and obtained high coverage genome sequence data by paired-end Illumina shotgun reads for almost all protein coding genes in 65 isolates. Excluding large sub-telomeric gene families (Var, rifin and stevor) and after masking repeat sequences, we obtained high quality data for >70% of the coding sequence in most genes, including 2853 that contained at least 3 single nucleotide polymorphisms and thus sufficient information for analysis. From analysis of summary indices generally useful for identifying outlier loci, we identified 241 gene loci (5% of genome) with positive signatures of balancing selection. Our results were concordant for a majority of genes previously studied by capillary re-sequencing in independent population samples and we further identified candidate loci with even more extreme evidence of balancing selection in a large number of merozoite specific genes, now prioritized for functional study and vaccine candidacy.

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THE TRANSCRIPTION FACTOR T-BET REGULATES PARASITEMIA AND PROMOTES PATHOGENESIS DURING *PLASMODIUM BERGHEI* ANKA MURINE MALARIA

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$CD4^+$ T cells are required for the pathogenesis of experimental cerebral malaria (ECM) during the induction phase of disease in mice. Using the *Plasmodium berghei* ANKA murine model of ECM and mice deficient for the transcription factor T-bet (the master regulator of Th1 cells) on the C57BL/6 background, we demonstrate that while Th1 $CD4^+$ T cells play a role in the regulation of parasite burden, they also promote the pathogenesis of ECM possibly by invoking a robust proinflammatory response. T-bet deficient mice had higher parasitemia than wildtype controls during the ECM phase of disease (17.7 ± 3.1% verses 10.9 ± 1.5%). In addition, while 100% (10/10) of wildtype mice developed ECM by day 9 post-infection, only 30% (3/10) of T-bet deficient mice succumbed to disease during the cerebral phase of infection ($p<0.0001$). In depth analysis of immune cells and cytokines is currently being performed to better understand how Th1 $CD4^+$ T cells mediate their protective as well as pathogenic functions during malaria infection.

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CHEMOKINE LEVELS DURING PREGNANCY MALARIA ARE ASSOCIATED WITH REDUCTION IN BIRTHWEIGHT

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In malaria endemic areas, first time mothers are highly susceptible to malaria infection. The main risks associated with pregnancy malaria (PM) are reduction in birthweight and maternal anemia. During normal pregnancy, the placenta displays a bias toward type 2 cytokines. However, pregnancy malaria shifts the balance from type 2 to type 1 cytokines. In our previous studies, type 1 cytokines like IFN-γ and TNF-α were associated with reduction in birthweight. Here, we examined placental levels of inflammatory chemokines and related their levels with pregnancy outcomes. CXCL9 and CXCL13 were significantly higher among malaria-infected pregnant women of all gravidities. However, high chemokine levels negatively correlated with birthweight among first time mothers only. CXCL9 is one of the chemokines induced by IFN-γ previously shown to increase during PM. The results presented here further expand our understanding of the mechanisms leading to poor pregnancy outcomes.

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CLINICAL CORRELATES OF MAGNETIC RESONANCE IMAGING IN PEDIATRIC CEREBRAL MALARIA

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Elucidating the pathogenesis of pediatric cerebral malaria (CM) has been a challenge for clinicians and pathologists because the relative contributions of sequestered parasites, metabolic derangements, seizures, and co-infections have been difficult to determine. The routine clinical characterization of pediatric CM patients in Blantyre includes direct and indirect ophthalmoscopy (to identify patients with malaria retinopathy), routine electroencephalography (to describe seizure semiology and identify subclinical seizures), and blood and cerebrospinal fluid cultures (to identify co-infections). The recent addition of neuroimaging, using a 0.35T Signa Ovation magnetic resonance imaging (MRI) machine (General Electric), has facilitated the recognition of previously unrecognized neuroradiologic features. Two-hundred thirty one patients with clinically defined cerebral malaria have undergone MRI imaging; the overall mortality rate was 13.4%. Of those scanned, 169 had malaria retinopathy, and 28 (16.6%) of these died. When compared to patients with retinopathy-negative CM (e.g., patients with a non-malaria cause of coma and parasitemia), the retinopathy-positive CM patients were more likely to have cerebral edema, and signal changes in the periventricular white matter, the basal ganglia and the corpus callosum. Frequently, rapid and significant changes were observed within 24-72 hours of admission. Seventy-three CM patients with retinopathy (43%) had evidence of severe cerebral edema (effacement of sulci, evidence of herniation) on admission. Twenty-eight (37%) of these patients died. There were no deaths in retinopathy-positive CM patients without evidence of severe brain swelling. These findings suggest that cerebral edema is an important feature in children with cerebral malaria, and that measures targeted at quickly reducing brain swelling may have an impact on survival.

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TRANSCRIPTIONAL PROFILING OF *PLASMODIUM FALCIPARUM* PARASITES FROM PATIENTS WITH SEVERE MALARIA: PARASITEMIA DRIVEN EXPRESSION AND UNIQUE BIOLOGICAL STATES

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The transcriptional biology of *Plasmodium falciparum* parasites from severe malaria *in vivo* has not been described. We collected peripheral blood from children meeting the clinical case definition of cerebral malaria and performed whole genome *ex vivo* transcriptional profiling for *Plasmodium falciparum* using a custom designed Affymetrix array. After normalization and clustering, two distinct biological clusters were observed that strongly correlated with the level of parasitemia observed in the patients at the time of collection. The low parasitemia cluster A (a heterogeneous mixture of profiles) showed upregulation of cell adhesion molecules, the trophozoite and gametocyte stages of the parasite, glycolysis, and cell cycle/DNA replication while the high parasitemia cluster B (a collection of very similar homogeneous profiles) demonstrated upregulation of genes and pathways associated with the ring stage of the parasite, activation of the ubiquitin pathway, and cytoplasmic ribosome translation. Comparisons with previous *ex vivo* transcriptional data in Senegal (predominantly from low parasitemia patients) showed expected overlap with cluster A while cluster B appears to be similar but unique to the Malawi patients. When ~1400 expression experiments from yeast were projected onto the Malawi metagenes, a subset of the Cluster B parasites could not be matched with known yeast biology although overlap with existing drug experiments were found. Correlation with the available clinical data demonstrated higher hematocrit in cluster A while the cluster B showed a higher use of mosquito repelling bed nets suggesting a link to low parasitemia. Lastly, we compared retinopathy the cluster A and B patients with retinopathy (and thus cerebral malaria) to all of the retinopathy negative patients (a heterogeneous mixture of diagnoses) and found the same association with parasitemia but also with white cell count (borderline significant); there were, however, a set of clinical differences that were distinct for retinopathy positive and not explained by parasite expression biology including low platelet count (expected), increased HIV rate, low hematocrit/hemoglobin (expected), low glucose (expected), high rate of bednet use, male gender (expected), and higher rate of lumefantrine/artemether use.

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MIXED INFECTION (*PLASMODIUM FALCIPARUM* AND *P. VIVAX*) DOES NOT REDUCE THE SEVERITY OF THE *P. FALCIPARUM* MALARIA - A STUDY FROM BIKANER, NORTHWEST INDIA

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Malaria remains a major health concern in tropical and subtropical countries. The last decade has witnessed a changing pattern of presentations and complications across the world. Independently *Plasmodium falciparum* malaria as well as *P. vivax* are known to cause of severe malaria and multiple organ failure whereas the effect on severity in mixed infection is conflicting. This prospective observational study describes the severity pattern and spectrum of severe manifestation (SM) of *P. falciparum* (Pf) mono infection as well as mixed infection with *P. vivax* (Pv+Pf) and study the effect of concomitant *vivax* infection on severity. This observational study included 887 adult patients amongst them 781 (88.04%) were Pf, and 106 (11.95 %) were mixed infection admitted in medical wards of S.P. Medical College and Associate Group of Hospitals, Bikaner, India from Sept. 2007 to December 2010. Species diagnosis was done by PBF and RDT. PCR confirmation on 100% patients revealed >96% accuracy. As defined in WHO criteria (2000) severe malaria was detected in 508 (57.27 %) patients with relative risk in Pf as 56.72 % (443/781) and in mixed infection as 61.32 % (65/106) [Pf - mixed infection p <0.0001)]. Hepatic dysfunction was the major SM (45.60% in Pf and 49.23% in mixed infection), followed by severe anemia (39.95% in Pf and 35.38% in mixed malaria), renal failure (12.19% in Pf and 10.77% in mixed malaria), cerebral malaria (8.80% in Pf and 10.77% in mixed malaria) and ARDS (0.68% in Pf and 0.0% in mixed malaria). Thrombocytopenia was observed in 46.05% in Pf and 50.77% in mixed infection. Multi organ dysfunction was 43.12% in Pf and 63.08% in mixed infection (p<0.0001). Mortality in indoor patients was 2.43 % (19/781) in Pf and 5.66% (6/106) in mixed infection. Inter group differences in all manifestations were statistically not significant but in case of MODS it was highly significant (p<0.0001). In conclusion, mixed infection does not reduce the severity of Pf malaria; rather it increases the severity and mortality.

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DISSECTING THE *PLASMODIUM FALCIPARUM* EVASION OF THE MOSQUITO IMMUNE SYSTEM

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Anopheles gambiae, main vector of malaria in Africa, varies in its susceptibility to different *Plasmodium falciparum* lines. It has been found that the mosquito innate immune system through the TEP1 pathway is able to eliminate *Plasmodium* parasites. We studied the role of the TEP1 pathway in determining the susceptibility of *Anopheles gambiae* to different *P. falciparum* lines. The *A. gambiae* L3-5 presented high susceptibility to *P. falciparum* 3D7, NF54 and GB4 lines, but it encapsulated in the midgut 97% of *P. falciparum* 7G8 parasites. dsRNA mediated knock down of components of the TEP1 pathway (TEP1, LRIM1, and APL1) rescued the 7G8 line from encapsulation, indicating that this pathway determines its elimination. On the other hand, dsRNA silencing of TEP1 and LRIM1 did not change significantly the infection of L3-5 mosquitoes with the *P. falciparum* NF54 line, indicating that this line evades the TEP1 pathway. Coinfection of *A. gambiae* L3-5 with a *P. falciparum* line that is encapsulated (7G8) and one that infects the mosquito effectively (3D7) led to mosquito midguts with both live and encapsulated parasites without any outcome dominating over the other. This indicates than even with activation of the TEP1 pathway by a *P. falciparum* line, a genetically different parasite can still evade it, suggesting that the evasion mechanism is parasite specific and not systemic in nature. Infecting with *Plasmodium* an extensive genetic cross of *A. gambiae* L3-5 with G3 *Plasmodium* susceptible mosquitoes we found that while the encapsulation of *P. berghei* is still mainly determined by the TEP1 alleles present in the mosquito, the *P. falciparum* 7G8 is no longer encapsulated, indicating that there are other factors besides the TEP1 allele that determine whether *P. falciparum* is eliminated by the mosquito immune system. The evasion of the *A. gambiae* immune system may be the result of adaptation of the parasite to the mosquito and has important implications for understanding disease transmission and the possibility of controlling it by enhancement of the TEP1 pathway in the mosquito.

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ANCESTRAL CHROMOSOMAL ARRANGEMENT AND REVISED PHYLOGENY OF THE *ANOPHELES GAMBIAE* COMPLEX

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The *Anopheles gambiae* complex consists of seven morphologically indistinguishable sibling species. Members of the complex have different geographical distribution, behavior and vectorial capacity, however, the phylogenetic relationship among the members of the complex is not resolved. For a long time *An. quadriannulatus* was considered ancestral because of its central position relative to other species, later analysis of the 2La inversion revealed that this arrangement is ancestral and has a unique origin. As a result *An. gambiae*, *An. arabiensis* or *An. merus* that carry this inversion could be closest to ancestral species. In this study, the breakpoints of 2Rop, the inversions fixed in *An. merus,* have been analyzed. Genes adjacent to breakpoints were identified by screening the *An. merus* Lambda Dash II phage library, Fluorescent *In Situ* hybridization (FISH), and Mate- Pair genome sequencing of *An. merus*. Proximal breakpoint of 2Ro inversion was obtained by screening the *An. merus* phage library and the distal breakpoint of 2Ro inversion was obtained by Mate -Pair sequencing of *An. merus* genome. FISH was done with the genes adjacent to breakpoints. The gene structure of inversion breakpoints was compared with several outgroup species including *An. stephensi, An. nili, An. moucheti, An. sinensis, Aedes aegypti* and *Culex quinquefasciatus.* The same gene arrangement at the 2Ro breakpoints was found in outgroup species, confirming the ancestral status of the 2Ro inversion. FISH also showed the same gene arrangement in 2Rp breakpoints in *An.gambiae* and outgroup species indicating that 2Rp+ arrangement is ancestral. Based on our data, we revised the chromosomal phylogeny of *An. gambiae* complex. We conclude that since 2La, 2Ro and 2Rp+ arrangements are ancestral; a hypothetical species that contained all these arrangement could be ancestral. It could have given rise to *An. merus* by obtaining the 2Rp arrangement and gave rise to *An. gambiae* by acquiring the 2Ro+ arrangement. The data suggest that the major vector of malaria in the world is more closely related to ancestral species and evolution shows that vectorial capacity can be lost in other members of the complex.

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A CONTINENT-WIDE MICROSATELLITE SURVEY REVEALS FURTHER COMPLEXITIES IN THE POPULATION STRUCTURE OF *ANOPHELES GAMBIAE* S.S. (*DIPTERA: CULICIDAE*)

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The major malaria vector *Anopheles gambiae s.s.* displays strong population subdivision across sub-Saharan Africa. In West Africa, two molecular forms have been described and are considered units of incipient speciation, albeit with varying levels of inter-form gene flow along their sympatric distribution range. However, studies on molecular form differentiation often analyzed samples from relatively few localities or regions. To provide an overall picture of the population structure of *An. gambiae s.s.* in west Africa, we have genotyped 25 samples, obtained mostly by indoor resting collections, from 12 African countries for 13 microsatellites on chromosome-3. Our area-wide results confirm a clear genetic differentiation between M and S forms using loci outside genomic regions of highest divergence. Furthermore, both Bayesian clustering and principal components analyses revealed further population substructuring in the M-form, with samples from Western Africa (from The Gambia to Nigeria) forming a distinct genetic cluster from those of West-Central Africa (from Cameroon to Angola). This subdivision is likely to be associated with the Forest-savannah ecosystem transition coupled with the accumulation of polymorphic chromosomal inversions in this vector species.

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CHROMOSOMAL AND MOLECULAR CHARACTERIZATION OF *ANOPHELES GAMBIAE* M AND S MOLECULAR FORMS IN A SECONDARY CONTACT ZONE AT THE WESTERNMOST EXTREME OF THEIR RANGE

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Throughout west and central Africa, *Anopheles gambiae* M and S molecular forms are characterised by largely overlapping geographical/ temporal distributions and high levels of reproductive isolation. Floating paracentric inversions on chromosome-2, probably involved in ecological adaptation to marginal sub-niches, are shared by the two forms, although with different frequencies of alternative inverted arrangements, reaching the highest level of inter-form differentiation in northern savannah areas. At the westernmost extreme of their range, however, a secondary contact zone between M and S forms has been recently revealed based on the finding of putative M/S hybrid frequencies higher than in the rest of the form range (i.e. 3-7% in The Gambia and >20% in Guinea Bissau). We here report the first results of the karyotyping of M and S populations collected in two west to east transects: one along the Gambia river in The Gambia and eastern Senegal and one from the capital city eastwards in Guinea Bissau. The results show that in coastal and central Gambian areas, as well as in the Guinean capital city area, M and S populations are found in sympatry and share the same chromosomal polymorphisms based on 2Rd and 2La inversion. On the other hand, in Senegalese sampling sites, S-form is largely predominating over M-form and is characterised by increasing frequencies of 2Rj, 2Rbk and 2Rcu inverted arrangements, while in eastward Guinean sites, S-form characterized by increasing frequencies of 2Rj and 2Rb is virtually the only form found. We will couple these results with those obtained from an extensive microsatellite analyses carried out on the same samples in order to provide a more detailed picture of the genetic differentiation between the two molecular forms in this area of secondary contact at the westernmost extreme of their range and to speculate on the genetic adaptive mechanisms allowing *An. gambiae* s.s. great ecological flexibility.

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WING SIZE DIFFERENTIATION BETWEEN THE INCIPIENT SPECIES OF *ANOPHELES GAMBIAE* S.S. AND ITS POTENTIAL ROLE IN ASSORTATIVE MATING

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Anopheles gambiae s.s., one of the most important malaria vector species in Africa, has been subdivided into two molecular forms, M and S, which are thought to represent incipient species. The two forms have varying levels of phenotypic and genetic divergence in different locations within their range. Wing beat frequency may provide the necessary phenotypic information required to explain the assortative mating observed between forms. Because wing size relates directly to both wing beat frequency and individual fitness we investigated the potential that wing size may vary with environmental and genetic variation in *An. gambiae* s.s in west Africa. We assessed the size of wings (wing length and wing width) from female mosquitoes collected at sites in Mali where a rarity of hybrids indicates strong between-form assortative mating, as well as Guinea-Bissau where high levels of hybridization suggest reproductive isolation between forms has broken down. We observed a significant difference in length and width of the wings between Guinea-Bissau and Mali. Guinea-Bissau mosquito wings were significantly smaller than those from Mali, regardless of molecular form. While we found no significant difference in mean wing length between molecular forms, we did find the S form to have significantly larger wing widths than the M form in Mali. By contrast, we did not observe this difference in Guinea-Bissau where the rate of hybridization in the field is high and assortative mating appears to be distorted. These data represent the first documentation of a morphological difference within *An. gambiae* s.s., between the two molecular forms. The significant difference observed between molecular forms for wing size, in an area of low hybridization and the lack of difference in an area of high hybridization supports the hypothesis that wing size and hence wing beat frequency may confer the necessary phenotypic information to account for the assortative mating observed with molecular data, between the molecular forms of this species.

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GENETIC ISOLATION BETWEEN *ANOPHELES MELAS* POPULATIONS

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Anopheles melas is found breeding in brackish waters along the coast of West-Africa. Because its distribution is limited to coastal regions, it is not often considered an important malaria vector, even though it is frequently the most abundant vector in those locations were it is present. Therefore, the species has not been studied well and little is know about its population structure. On Bioko Island, Equatorial Guinea, *An. melas* is an important vector. It is dominant in several locations where it is responsible for a high entomological inoculation rate. Malaria vector populations on Bioko have been targeted by anti-vector interventions implemented under the Bioko Island Malaria Control Project (BIMCP). As part of an operational research project under the BIMCP, we have investigated the population structure of *An. melas* on Bioko and mainland Africa to determine the level of migration between various populations on the mainland and Bioko Island. We have analyzed microsatellite data and mtDNA from 11 *An. melas* populations across West-Africa. We found that *An. melas* populations for the most part cluster into three distinct groups with a very high level of genetic differentiation between them. Populations on Bioko Island are almost completely isolated from the mainland. Additionally, mainland populations are divided into two distinct groups which do not share mtDNA haplotypes and little genetic exchange is evident in the microsatellite data. In fact, the level of mtDNA differentiation between the two mainland *An. melas* clusters is on par with that between *An. melas* and *An. gambiae.* These results indicate that *An. melas* on the mainland may consist of two previously undescribed species. Additionally, in contrast to its sister species *An. gambiae,* little or no migration exists between *An. melas* populations on the African mainland and Bioko Island.

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GENOME-WIDE PROFILING OF CIRCADIAN AND LIGHT-REGULATED GENE EXPRESSION OF THE *ANOPHELES GAMBIAE* MOSQUITO REVEALS DAILY RHYTHMS IN METABOLISM, DETOXIFICATION, IMMUNITY AND SENSORY PROCESSES

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Anopheles gambiae, the primary African mosquito vector of malaria, exhibits numerous rhythmic behaviors including flight activity, swarming, mating, host seeking, egg laying and sugar feeding. However, little work has been performed to elucidate the molecular basis for these daily rhythms. To study how gene expression is globally regulated by light and circadian mechanisms, we have undertaken a microarray analysis of *A. gambiae* under light:dark cycle (LD) and constant dark (DD) conditions. Adult female mosquitoes were collected every 4 hr over 48 hr and samples were processed with microarrays. Using a cosine-wave fitting algorithm, we identified 1293 and 600 rhythmic genes with a 20-28 hr period length in the head and body under LD conditions, representing 9.7 and 4.5% of the *A. gambiae* gene-set (www.nd.edu/~bioclock). A majority of these genes was specific to heads or bodies. Through examination of mosquitoes under DD conditions, we reveal that rhythmic programming of the transcriptome is dependent upon an interaction between the endogenous clock and extrinsic regulation by the LD cycle. A sub-set of genes was rhythmically expressed under both environmental conditions, including the canonical clock components. A majority of genes had peak expression clustered around the day/night transitions, anticipating dawn and dusk. Genes cover diverse biological processes such as transcription/translation, metabolism, detoxification, olfaction, vision, cuticule-regulation and immunity. For example, 33% of cytochrome P450 genes are rhythmically expressed, including CYP6Z1, CYP6P3 and CYP6M2 that are implicated in pyrethroid resistance. In olfaction, genes encoding odorant binding proteins and the olfactory coreceptor OR7 are rhythmically expressed, as are components of the visual transduction pathway, suggesting that daily changes occur in sensory perception and in the ability of the mosquito to detect host-cues. This study highlights both the fundamental roles that the circadian clock and light play in the physiology of *A. gambiae*, and suggests novel targets for intervention.

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THE RESEARCH ON THE SCHISTOSOMIASIS TRANSMISSION DYNAMIC MODEL BASED ON ARTIFICIAL INTELLIGENCE

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Schistosomiasis is one of the most prevalent parasitic diseases worldwide, with 207 million people infected in 76 countries. In China, *Schistosoma japonicum* is the species that causes human infection and disease in endemic areas. Although over 60 years' control efforts has successfully bring down the prevalence, further reduction and eradication of the disease remain difficult due to the complexity of life cycle of the parasite as well as the impact of individual behavior on transmission dynamics. To better predict the impact of control policies including chemotherapy scheme and vaccination at the low-endemic setting, here we present a stochastic model (SjCA-Q) based on a revised cellular automata model using Q-learning algorithm as the artificial intelligence to describe the transmission dynamics of human schistosomiasis japonica in an endemic area in China. This model includes the process of pathogen invasion from exposure to worm development and worm death when the infection is cleared; it also incorporates seasonality of infection in the endemic field as well as the stochastic behavior of each individual. Q-learning algorithm is used for rules self-learning in the model. For simulation, we used data collected from a two-year longitudinal study conducted in Jiahu village on the southeastern shore of Poyang Lake in Jiangxi Province. We applied our model in evaluation of several current control strategies for schistosomiasis in China. A multi-dimensional evaluation system is used to judge the simulate result. Our model can effectively simulate the transmission process. The Q-learning algorithm enabled the model self-learn the uncertain rules in disease transmission model and significantly improve the simulation effect. Chemotherapy should cover no less than 85 percent of the *Schistosoma japonicum* infected population, and implemented twice a year in the low-endemic community to guarantee an effective control. In conclusion, it is anticipated that our SjCA-Q transmission model can serve as a tool for understanding schistosomiasis transmission dynamics and thus the knowledge learnt from modeling would be prerequisite for focusing and improving schistosomiasis control at the local level.

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NEW AND IMPROVED IMMUNOLOGICAL ASSAYS FOR DIAGNOSIS OF *SCHISTOSOMA MANSONI*

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Increasing populations and human migration are contributing factors in the observed increases of *Schistosoma mansoni* in new areas of southeastern Brasil. Control constraints include the lack of diagnostic methods with high sensitivity. We initiated a study in southeast Brasil to develop and improve diagnostic methods for *S. mansoni*. Individuals from 3 endemic areas and, non-endemic rural tourists with acute disease were selected as sera and feces donors. Specificity/sensitivity of new diagnostic methods tested were compared to results obtained from 20 Kato-Katz prepared slides from 3 different fecal samples collected on different days for each individual. Miracidial hatching from each fecal sample was also measured. We evaluated the efficiency of egg versus worm antigens using two immunosorbant assays for IgG detection based on the antigens being highly immunogenic and easily obtained. Both new tests presented a kappa index of 0.46. The first new assay had sensitivity/specificity of 75% with a cut off of 0.31. The second new assay had 100% and 97% sensitivity/specificity respectively with a cut off of 0.18. Currently, diagnosis of pre-patent schistosome infections is difficult due to non-specific symptoms. Therefore, we standardized a new IgG detection assay using schistosomula antigen. Data showed excellent agreement of kappa index (0.82) and 91% and 53% of sensitivity/specificity with a cut off of 0.29, including all chronic patients with low parasite load (1-200 epg/feces). The method was also able to detect high antibody titers in sera of non-endemic patients after 7 days of the infection. Although antibody-based methods suffer from low sensitivity, especially for acute phase, we were able to identify positive cases for endemic and non-endemic patients. Finally, we developed a direct method for use with acute and chronic phase diagnosis that differentially detects *Schistosoma* antigens from other worms using low amounts of human sample.

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ASSESSMENT OF SUBTLE MORBIDITY DUE TO SCHISTOSOMIASIS AMONG SCHOOL CHILDREN: THE SCORE PROJECT IN KENYA

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Chronic *Schistosoma mansoni* infection is associated with liver fibrosis, stunting, wasting, anemia, exercise intolerance, and decreased quality of life. Much of the morbidity associated with schistosomiasis is subtle, making it difficult to quantify disease burden. Thus, the effectiveness of control programs may be underestimated. The Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) project is a five year multi-national study designed to evaluate optimal control strategies for *S. mansoni* associated morbidity. We present the merged baseline results of morbidity assessments in two cohorts from Kenya. A total of 817 children aged 7-8 years near Kisumu, Kenya were randomly selected from 12 schools with *S. mansoni* prevalence ≥25%. Stool was collected to test for *S. mansoni* infection and intensity, and soil-transmitted helminth (STH) infection. Blood was tested for malaria and anemia status. Ultrasound data were collected. Stool results from 620 persons show a prevalence of *S. mansoni*, *Trichuris trichiura*, *Ascaris*, and hookworm of 66.2% (62.4-70.0), 15.7% (13.1-18.8), 12.0% (9.7-14.9), and 5% (3.5-7.1), respectively; 26.8% (23.4-30.4) were positive for any STH, and 73% (69.4-76.4) were positive for at least one helminth. Malaria infection was found in 8.0% (5.8-11.0) of the 424 tested. Anemia was present in 48.3% (44.9-51.8) of the 801 children tested- 40% (36.6-43.4), 7.6% (6.0-9.7), and 0.8% (0.3-1.7) with mild, moderate, and severe anemia, respectively. Abdominal ultrasounds showed abnormal liver texture patterns in 23.6% (20.7-26.7) of the 781 children tested. Univariate analysis of infection and anemia showed statistically significant associations with *S. mansoni*, OR 1.5 (1.04-2.1), and any helminth infection, OR 1.46 (1.01-2.12) only. In this cohort of 7-8 year old Kenyan children, *S. mansoni* and STH infections were highly prevalent and associated with anemia. Dataset completion and multi-variate analyses, including exercise tolerance, anthropometric, and quality of life measures, are forthcoming.

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ASSESSMENT OF QUALITY OF LIFE AS A TOOL FOR MEASURING MORBIDITY DUE TO SCHISTOSOMIASIS AND THE IMPACT OF TREATMENT

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Schistosomiasis control programs are designed to reduce morbidity associated with the infection. In areas endemic for *Schistosoma mansoni*, change in prevalence, based on stool examination, is the primary

method used for monitoring the efficacy of treatment. However, this assessment is often difficult to conduct and may not reflect the health benefits gained. Because schistosomiasis can persist for many years causing unrecognized morbidity, a proposed approach to assess program impact is to use questionnaires that capture measures of quality of life. To evaluate whether the short form WHO quality of life assessment (WHOQOL-BREF) is useful to measure the benefit of treating *S. mansoni* infections, adults from a highly endemic area (> 75% prevalence) who had no recollection of prior treatment with praziquantel were enrolled. Prior to treatment, the WHOQOL-BREF was administered to non-pregnant, consenting participants who were evaluated for schistosomiasis by stool exam, serum antibody levels, and presence of circulating cathodic antigen (CCA) in urine. Additionally, participants were tested for infection with malaria, soil transmitted helminths and HIV. At baseline, there was no association between schistosome infection status or intensity of infection and quality of life. Urine CCA levels were significantly reduced within 2 days of treatment. Six months after treatment, the WHOQOL-BREF was administered again and stool and urine samples collected. There was a significant reduction in both prevalence and intensity of infection and quality of life significantly improved compared to baseline. However, persons who did not have detectable *S. mansoni* infections at baseline demonstrated similar improvements in their WHOQOL-BREF scores as did persons infected at baseline. Similarly, there was no relationship in the baseline intensity of infection and the improvement of reported quality of life. Thus, in areas of high prevalence and intensity of schistosomiasis, the WHOQOL-BREF may not be able to specifically detect the benefits of mass drug administration programs.

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COMPARISON OF QUESTIONNAIRE-BASED PRAZIQUANTEL TREATMENT HISTORY WITH HEMATURIA AMONG SCHOOL CHILDREN

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National schistosomiasis control programs often report the number of praziquantel (PZQ) treatments distributed as a measure of program output, along with coverage calculations that use a host of different denominators, including total population, eligible population, or census population. It has been proposed that reported coverage by programs be independently confirmed through individual responses in drug coverage surveys. However, response bias and/or recall bias have been proposed as major limitations of such surveys. As part of a 2010 evaluation of ongoing PZQ distribution to school-aged children in Plateau and Nasarawa states of north-central Nigeria, we randomly selected 482 school-children 10-14 years of age from 12 schools across two LGAs receiving annual PZQ treatment to reassess hematuria using urine reagent dipsticks. Baseline mean hematuria in these village in 2008 was 34.1%. All students were asked whether they took or did not take PZQ for schistosomiasis in the last year. PZQ tablets were shown to the children upon assessment. Overall, 13.3% (6.9-19.7%) tested positive for hematuria. Drug coverage as reported by surveyed children was 64.5% (47.3-81.8%). After controlling for sex, baseline community prevalence and reported knowledge of schistosomiasis, the odds of having hematuria were 2.1 (95%CL 1.13-3.9) times higher among children reporting not taking PZQ than children reporting taking PZQ. These results suggest school children provide reliable history of PZQ treatment. National programs should consider implementing similar evaluation surveys in schools to monitor drug coverage and impact.

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COMMUNITY-BASED INTERVENTION TO REDUCE INCIDENCE OF SCHISTOSOMIASIS THROUGH TREATMENT AND HEALTH EDUCATION IN RURAL AFRICA

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Schistosomiasis is a chronic parasitic infection affecting millions of Africans, acquired in fresh water via snail host. This is particularly precarious for inhabitants of rural areas that depend on the local fresh-water source. Due to limited access to hospitals and education, lakeside villagers are untreated, and simply unaware of precautions against schistosomiasis. By providing annual on-site screening, education and treatment, the incidence of Schistosomiasis can be reduced. Since 2006, a longitudinal study has been carried out in Minigo, a village along Lake Victoria. Minigo (pop. 3635) was educated on the practices exposing them to Schistosomiasis and how to modify daily activities to minimize this. On location screening for *S. haematobium* and *S. mansoni* was provided through physical exam and fecal and urine sample testing. Praziquantel 40 mg/kg was prescribed to those who tested positive and prophylactically for fisherman. For those with complications, an ultrasound was provided to determine need for other treatment. For those positive for other infections, appropriate treatment was given. In 2010, this method was introduced into a second village, Masonga (Pop. 2000). In Minigo, the incidence was 30% in 2007, 14% in 2009, and 10% in 2010, yielding a 67% decline. Masonga (Pop. 2000), providing a basis for comparison, had an incidence of 20% in 2010. While all of Minigo and Masonga had access, only 5% and 10%, respectively, elected to be screened and treated. Accordingly, the prevalence of Schistosomiasis can be effectively reduced by providing access to treatment and preventative education at the community level. Masonga, providing a baseline rate two times higher than that of Minigo reiterates the value of this intervention. Increasing participation is needed for the eradication of Schistosomiasis, but the factors influencing decisions to seek community-based disease prevention has not yet been determined. If these results are confirmed with the continuation of this study, this method of cooperative intervention will provide a feasible model for Schistosomiasis reduction in lake-dependent villages in rural Africa.

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BILHVAX, A VACCINE CANDIDATE AGAINST SCHISTOSOMIASIS

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The development of an efficient vaccine against human schistosomiasis represents a major challenge for the improvement of health in many developing countries where continuous re-infection point to the necessary development of alternative strategies to chemotherapy. In schistosomiasis, where parasite eggs laying in the tissues is the exclusive cause of pathology and the elimination of eggs in nature is the source of transmission, inhibition of parasite fecundity might represent a way to prevent the deleterious effects of these chronic infections in man. The concept to target by vaccination the cause of the pathology rather than the parasite itself would provide a potent tool to control a major chronic infection. In *Schistosoma haematobium* infected children, clinical trials (Phases I-II) provided evidence of a safe and immunogenic molecule inducing in man a profile of immune response which seems in accordance with the experimental models describing the effect on inhibition of female worm fecundity and egg viability. On this basis, Inserm proposed to develop efficacy phases (Phases III). As a whole, the first step of clinical trials of the first vaccine candidate against human schistosomiasis Phase III trial, self-contained, randomized, double blind, in two parallel groups receiving 3 primo-vaccinations and a boost has been stated in March

2009 for a three-year trail. During this trial, one group of *S. haematobium* infected children (6 to 9) receives "Bilhvax", the other one, placebo. Both groups were treated with Praziquantel. The aim of the trial is to evaluate efficacy and safety of the therapeutic vaccine candidate Sh28GST in association with Praziquantel for prevention of clinical and parasitological recurrences of *S. haematobium* infection in children. These trials which are performed in already established clinical platform in Senegal and could be easily extended to other schistosome sp infections, represents a crucial step in innovative approaches to solve persistent problems of these chronic parasitic infections.

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PLASMODIUM FALCIPARUM HISTONES INDUCE ENDOTHELIAL PRO-INFLAMMATORY RESPONSE AND BARRIER DYSFUNCTION

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Severe *Plasmodium falciparum* infection is associated with endothelial activation and permeability that are important determinants of the outcome of the infection. How endothelial cells become activated is not fully understood but is believed to be either a response to cytokines from leukocytes and/or a direct effect of parasite components. In this study, we demonstrated that *P. falciparum* sonicates directly stimulated the production of IL-8 and other inflammatory mediators by primary human dermal microvascular endothelial cells through a signalling pathway that involved the Src family kinase Lyn and p38 MAPK. The active parasite component was identified as acid soluble proteins (HeH) of which histones were a major constituent. The role of histones was confirmed by abrogation of the stimulatory effect of HeH by histone-specific antibodies and the use of recombinant *P. falciparum* H3 (PfH3) and recombinant human H4. Confocal microscopy of methanol-fixed blood smears revealed that prior to schizogony, histones could be seen both inside and outside of nuclei of merozoites. The release of nuclear contents upon IRBC rupture was captured by live cell imaging using the cell membrane impermeable DNA stain Sytox Green, and was confirmed by detecting nucleosomes in supernatants of parasite cultures. HeH and recombinant histones also induced endothelial permeability through a charge-dependent mechanism that resulted in disruption of junctional protein expression and cell death. Recombinant human activated protein C cleaved HeH and PfH3 and abrogated both IL-8 production and increased permeability. Circulating nucleosomes of both human and parasite origin were detected in the plasma of patients with *falciparum* malaria, and correlated positively with disease severity. These results strongly support a pathogenic role for both host- and pathogen-derived histones in *P. falciparum* malaria.

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THE UNUSUAL TCA METABOLISM IN *PLASMODIUM FALCIPARUM*

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The role of tricarboxylic acid (TCA) metabolism in malaria parasites has been poorly understood for decades. Recent studies have shown that glutamine and glutamate are the primary carbon sources of the TCA metabolism and that the TCA "cycle" splits at α-ketoglutarate, forming a branched architecture. The functions of this unique TCA metabolism in *Plasmodium* parasites have been proposed to provide precursor for heme biosynthesis and to generate acetyl-CoA (instead of consuming it). To investigate this altered TCA metabolism, we have generated a series of knockout mutants via homologous recombination strategies. We have individually knocked out genes encoding α-ketoglutarate dehydrogenase (KDH), succinyl-CoA synthetase (SCS), succinate dehydrogenase (SDH) and isocitrate dehydrogenase (IDH). Furthermore, we generated KDH/SCS and SCS/SDH double knockouts. Remarkably, none of the knockout lines exhibited growth defects, suggesting these enzymes to be nonessential in the blood stage. In particular, the viability of the KDH/SCS double-knockout line indicates that succinyl-CoA production from the TCA metabolism is not essential. This result challenges the long held notion that TCA metabolism is necessary to provide the essential precursor (succinyl-CoA) for heme biosynthesis. Fumarate hydratase could not be deleted, suggesting it is essential. To determine the metabolic consequences of knocking out these TCA enzymes, we incubated the knockout parasites with U-^{13}C-glutamine medium. Oxidative and reductive TCA fluxes were quantified by measuring differential isotopic enrichment in various TCA intermediates by liquid chromatography mass spectrometry (LC-MS). ΔSCS line had no significant changes in the TCA metabolites. All ΔKDH and ΔSDH lines (single and double knockout) showed significantly diminished oxidative TCA flux when compared to wild type. As expected, reductive flux in the ΔKDH and ΔSDH lines was comparable to wild type. In contrast ΔIDH line showed significant impairment in both oxidative and reductive TCA fluxes, indicating little to no TCA cycle activity in these parasites. Surprisingly, such changes do not affect parasite survival, indicating a high degree of metabolic flexibility in malaria parasites.

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INTERFERON REGULATORY FACTOR 8 (IRF8) REGULATED PATHWAYS: ACTIVE ROLE IN PATHOGENESIS OF CEREBRAL MALARIA BUT REQUIRED FOR PROTECTION AGAINST TUBERCULOSIS

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Interferon Regulatory Factor 8 (Irf8) is a transcription factor that plays an important role in myeloid cell development and function. It is not only required for the development of monocytes, macrophage and dendritic cells, but also for transcription of intrinsic (microbicidal effector proteins) and extrinsic (IL-12p40) defense mechanisms expressed by these cells. The BXH2 mouse strain harbors an *Irf8*^{R294C} hypomorphic loss-of-function allele. BXH2 mice, or myeloid cells derived from them, are susceptible to many infections including *Mycobacterium bovis* BCG, *M. tuberculosis*, and *Legionella pneumophila*. Here, we observe that BXH2 mice are highly resistant to cerebral malaria caused by infection with *Plasmodium berghei* ANKA. Transcriptional profiling of BXH2 total brain RNA following *P. berghei* infection shows that CM-resistance in these mice is associated with the failure to transcriptionally activate interferon responsive inflammatory pathways. Of the 55 genes up-regulated (>2.5-fold) in cerebral malaria in an Irf8-dependent fashion (present in susceptible C57BL/6 and significantly reduced in BXH2 mice) following *P. berghei* infection, several of them are known to be play critical roles in Th1 polarization of immune response, including Irf1 and Stat1. Seventeen of these genes were also up-regulated by a factors of >1.8-fold in C57Bl/6J mouse lungs infected with *M. tuberculosis*. 94% (16/17) of genes regulated by both infections contained an Irf8 binding site, as determined by chromatin immunoprecipitation in macrophages followed by genome-wide hybridization (ChIP-chip). Additional studies in Stat1, Irf1 and If knockout mice confirmed that disruptions in this pathway confer both CM-resistance and susceptibility to tuberculosis. Our findings indicate that a robust Th1 response plays an important detrimental role in pathogenesis of cerebral malaria, while being required for protection

against mycobacterial infections. The Irf8-dependent transcriptional program identified here is shown to play a pivotal role in this response; Irf8 and/or members of this pathway may constitute a potential therapeutic target for intervention in cerebral malaria.

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ACTINOMYCETE-DERIVED INHIBITORS OF FILARIAL ASPARAGINYL-TRNA SYNTHETASE

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Aminoacyl tRNA synthetases (AARS) were one of the first new molecular targets embraced by the W.H.O helminth drug discovery program and are generally regarded as excellent therapeutic targets because; (i) they perform important primary and secondary transformations within eukaryotes including filaria and other human and veterinary parasites, (ii) are essential to parasite viability, and (iii) demonstrate primary and secondary structural heterogeneity. Among the AARSs AsnRS (asparaginyl-tRNA synthetase) is an excellent filarial target because (i) it is expressed in both sexes, adults, L1 (bloodborne microfilariae) and L3 (infective) larvae of *Brugia malayi* and *Wuchereria bancrofti*, (ii) is well-characterized biochemically and structurally, and (iii) recombinant *B. malayi* AsnRS is amenable to overexpression and use in high throughput bioassay-guided screening algorithms. As part of a drug discovery program targeting the *B. malayi* AsnRS we recently screened ~73,000 microextracts from a collection of 36,720 microbial strains for activity against *B. malayi* AsnRS. Natural product producers evaluated included members of the *Streptomyces, Deuteromyces, Aspergillus, Euteromyces, Peniclium, Malbranchea, Fusarium* and *Mucor* species. We recently reported that one of these strains, *Streptomyces* sp. 17944, produces a tirandamycin (TAM) with the ability to inhibit filarial AsnRS and rapidly kill adult worms. New data is now presented on optimization of experimental conditions that facilitate production of the TAM and application of these methods to discovery of additional filarial AsnRS inhibitors. We have identified fermentation conditions affording the TAM as the major product (with titers ~ 12 mg/L) and we have a developed an expedient genetic system for manipulation of TAM biosynthesis in *S*. sp. 17944. These results (i) demonstrate the feasibility of *in vivo* manipulation of TAM biosynthesis in *S*. sp. 17944 and (ii) ensure that sufficient amounts of the TAM can be produced and isolated for proposed follow up mechanistic and preclinical studies for consideration as a novel antifilarial.

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STANDARDIZING THE MEASUREMENT OF PARASITE CLEARANCE: PARASITE CLEARANCE ESTIMATOR

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The emergence and spread of resistance to antimalarial drugs threatens the efficacy of existing drug treatments. Although guidelines do not currently exist that define a consistent method of identifying resistance, monitoring times to parasite clearance has been widely used. The pharmacodynamic hallmark of the artemisinin derivatives is that they give more rapid parasite clearance than other antimalarials. Thus, accurate measurement of parasite clearance is critical to assess the emergence and spread of artemisinin resistance in *Plasmodium falciparum*, which has recently emerged in Western Cambodia. After starting antimalarial treatment, a lag phase of numerical instability, often precedes a fall in the parasite count. This complicates the parasite clearance rate estimation, introduces observer subjectivity, and may influence both the accuracy and consistency of results. To address this problem, a new approach to modeling clearance of parasites has been explored and validated. This model detects when a lag phase is present, allows the best model to be chosen from log linear, quadratic and cubic fits and calculates estimates of parasite clearance adjusted for this lag phase. Parasite measurements below the level of detection are accounted for in the estimation, and not excluded, as is usual per standard practice. Based on data from clinical studies in South East Asia in which existing frequent parasite count data were obtained, we present individual patient data examples for which the lag phase has been identified and discuss the effect it has on clearance rate estimates. Goodness of fit and residual plots are compared between standard linear regression method and our lag phase method. As part of WWARN efforts to make innovative approaches available to the malaria community, we have developed an open access automated informatics tool. This tool provides a more accurate, consistent and improved method of estimating both the parasite clearance rate and the lag phase. It could be used to detect early warning signs of resistance to artemisinin derivatives.

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PLASMODIUM FALCIPARUM CLEARANCE RATES IN RESPONSE TO ARTESUNATE IN MALIAN CHILDREN WITH MALARIA

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Artemisinin resistance, currently defined as a slow parasite clearance rate (CR) *in vivo*, was recently described in Cambodians with *Plasmodium falciparum* malaria. Studies have not yet reported parasite CRs from Africa, where artemisinin-based combination therapies (ACTs) are first-line treatments for *falciparum* malaria. In this study we measured parasite CRs in 132 Malian children aged 1-15 years presenting with uncomplicated *falciparum* malaria in 2010. We provided directly observed weight-based doses of artesunate (days 0, 1, 2) and amodiaquine (days 3, 4, 5) orally to these children, and counted the peripheral blood parasite density every 6h until it was zero. From plots of log-transformed parasite densities *vs.* time we calculated the half-life of parasite clearance (the time it takes for parasite density to decrease by 50%) and evaluated the effects of age, sex, ethnicity and red blood cell (RBC) polymorphisms (sickle HbS, HbC, alpha-thalassemia, G6PD deficiency). We isolated parasites from 46 of these children and measured their *ex vivo* response to artesunate and dihydroartemisinin (DHA) in a conventional drug response assay. The mean (± SEM) half-life of parasite clearance was 1.99h +/- 0.068h and ranged from 0.4h to 5.3h. A linear regression analysis showed that the half-life of parasite clearance decreased with age, predicting a 0.1h reduction in half-life for every 1-year increase in age. *Ex vivo* IC50s for DHA, but not for artesunate, correlated positively with half-life of parasite clearance. These data indicate that the artemisinin resistance phenotype is not present in our study population, consistent with the very recent introduction of this drug at our study site. In high-endemic areas, analyses of parasite clearance in response to artesunate should account for age as a covariate, since acquired immunity may increase the rate of parasite clearance *in vivo*. Studies of artemisinin resistance in low-endemic areas like Southeast Asia may be confounded by the patient's level of acquired immunity.

We are now using this *in vivo* model of parasite clearance in response to artemisinin to identify IgG responses that correlate with the removal of *P. falciparum*-infected RBCs during the course of a malaria episode.

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PARASITE CLEARANCE TIME FOLLOWING ORAL ARTESUNATE TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN MALI, WEST AFRICA

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Artemisinin based combinations (ACTs) are currently the first line therapy for uncomplicated malaria. *Plasmodium falciparum* resistance to artemisinins, measured by parasite clearance time (PCT), has been reported in South-East Asia. Few data on PCT are available from Africa where malaria transmission is high, the burden of malaria is highest and the use of ACTs has been scaled up in the past few years. From December 2010 to February 2011, 100 children from Bougoula-Hamaeau, Mali aged 1-10 years with uncomplicated malaria were enrolled and treated with seven days of directly-observed oral artesunate after parental consent. Thick and thin blood smears were prepared and read every 8 hours for asexual and sexual parasite counts until three consecutive slides were negative. Patients were followed actively and passively for 28 days following a standard protocol. Results were compared to data from a similar study conducted in the same village by the same study team and during the same months in 2002/04. In the per protocol analysis, the uncorrected adequate clinical and parasitological response (ACPR) rate of 96.7% and corrected ACPR of 100% measured in 2010-2011 (n=91) were similar to those measured in 2002/2004(98.6% and 100% for uncorrected and corrected ACPR, respectively). The proportions of patients who cleared parasitemia by 24 hours after treatment initiation were 36.0% (n=92) in 2010/11 and 31.9% (n=72) 2002/2004 (p=0.5). The median PCT in 2010-2011 was 32 hours. No PCT could be calculated with the 2002/2004 data because slides were read only at 24-hourly intervals. To our knowledge, this is the first estimate of PCT after curative artesunate therapy in an area of high transmission of *falciparum* malaria. Artesunate was highly efficacious and we therefore provide a baseline PCT that is required for the surveillance of the efficacy of artemisinins on *P. falciparum* isolates from sub-Saharan Africa.

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EMERGENCE OF ARTEMISININ RESISTANCE ON THE THAILAND-BURMA BORDER

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Resistance to artemisinin (ART) in *Plasmodium falciparum* is suspected from foci in Western Cambodia and is characterized by slower parasite clearance rates (CR) following treatment with artesunate. However, there is little information on the distribution of this phenotype elsewhere in SE Asia, and the genotypic basis of this trait is unknown. We measured parasite CR (using 6 hourly measures of parasitemia following ART treatment) in 1733 hyperparasitemia patients from 4 clinics on the Thai-Burma border between 2001-2010, and genotyped all parasite infections with 93 polymorphic SNPs. Parasite CR decreased significantly between 2001-10. While 4% of patients showed CR < 0.15 in 2001, this increased to 45% in 2010, compared with 78% in Western Cambodia. At the current rate of decline CR on the Thai-Burma border will be indistinguishable from current rates in Western Cambodia in <5 years. Partner drugs cannot explain these patterns. We observed the same temporal changes in a subset of 874 patients treated with monotherapy for >48hrs prior to mefloquine treatment. There was a minor influence of patient age, but waning population immunity due to declining transmission was also insufficient to explain the changes observed. To examine the role of parasite genetics we identified identical 93-locus parasite genotypes infecting multiple patients. We identified 158 multilocus parasite genotypes each infecting 2 - 14 patients. In 2001-4 29% of variation in CR was attributable to parasite genetics. Interestingly, the two parasites with slowest CR during this period, both from 2003, had identical 93-locus genotypes, suggesting the presence of ART resistant parasites 8 years ago. By 2007-10 parasite genetic factors explained 64% of variation in CR, consistent with increasing frequencies of parasite alleles conferring ART resistance. Both epidemiology and genetics provide compelling evidence that parasite CR is declining on the Thailand-Burma border and that this is explained by parasite genetic factors present in an increasing proportion of the parasite population.

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ARC3: A GENOME-WIDE ASSOCIATION STUDY OF THE GENETIC BASIS OF PARASITE CLEARANCE RATE FOLLOWING TREATMENT WITH ARTEMISININS

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In the wake of widespread resistance to chloroquine and antifolate drugs, artemisinin-based combination therapies (ACTs) have been adopted as the first line treatment for *Plasmodium falciparum* malaria in most regions of the world. The successful use of ACTs with insecticide-

treated nets to dramatically reduce the malaria burden in some areas has sparked renewed consideration of a global malaria eradication campaign. The emergence of artemisinin-resistant *P. falciparum* in parts of western Cambodia threatens the recent major global investment in ACTs and prospects for eradication. As part of the Artemisinin Resistance Confirmation, Characterization, and Containment (ARC3) pilot project, four clinical trials of artesunate curative therapy were conducted at two sites in western Cambodia where emerging resistance was suspected; on the Thai-Myanmar border, where prolonged parasite clearance times following artesunate-mefloquine treatment had also been reported; and in Bangladesh, where ACTs have not been used extensively and resistance was not suspected. Parasites collected during these trials were genotyped at approximately 8,000 single nucleotide polymorphisms (SNPs) using a molecular inversion probe SNP chip specific to *P. falciparum*. Regression and Random Forests were used to associate parasite genotypes generated from 331 samples with parasite clearance rates, adjusting for population structure, patient age, parasitemia at diagnosis, and study site. Statistically significant associations were observed between parasite clearance rate and SNPs on multiple chromosomes, with large clusters of significant SNPs on chromosomes 6, 9, 10, 11, 13, and 14. These results suggest that the phenotype of parasite clearance rate following treatment with artemisinins has a multigenic basis. Potential candidate genes within identified regions will be discussed.

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USING SCANS FOR SELECTION TO IDENTIFY GENES THAT UNDERLIE ARTEMISININ RESISTANCE

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Strong selection with antimalarials has generated several textbook examples of selective sweeps, in which resistance genes have spread through parasite populations purging genetic variation, while generating high frequency long haplotypes and elevated levels of geographical differentiation. Such molecular signatures of selection are readily detected from genomic data. We used a simple two-phase strategy to identify novel drug resistance loci that minimizes the multiple-testing penalties that result from brute force genome wide association approaches. First, candidate regions were identified by examining signatures of selection in genome-wide SNP data. Second, these candidate regions were directly screened for association with resistance phenotypes in a large sample of parasites. We applied this approach to emerging artemisinin resistance in SE Asia. Initially we compared the genomes of 91 parasites from W. Cambodia (slow clearance rate (CR)), Thailand (intermediate CR) and Laos (rapid CR) by genotyping 45K SNPs on a Nimblegen microarray. This identified 33 regions within the top 1% of genome wide values for FST and XP-EHH, statistics that measure differentiation between populations in SNP frequency and haplotype structure respectively. Encouragingly, these regions contained 4/5 known resistance loci (Pfcrt, dhfr, dhps and GTP cyclohydrolase I), confirming that the regions identified are enriched for loci involved in drug resistance. We are currently screening 96 SNPs within these 33 regions for direct association with CR in an independent sample of 768 unique single-clone parasites from the Thailand-Burma border for which CR has been determined using 6-hourly measurement of parasitemia following treatment with artesunate. Targetted genotyping of small numbers of SNPs reduces the multiple testing problem, allowing statistically powerful screens with relatively low sample size. We evaluate the success of this approach and report the loci identified.

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ARREST OF HEMOGLOBIN DIGESTION RENDERS MALARIA PARASITES INSENSITIVE TO ARTEMISININ

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Artemisinin-based regimens are the frontline treatment for resistant *Plasmodium falciparum* malaria. Artemisinin (Art) is thought to be activated by opening of its endoperoxide ring leading to potent cytotoxic radicals which may damage essential macromolecules in the parasite. However, the nature and origin of the activator and the molecular basis of the Art activity are matter of debate. To better characterize the antimalarial action of Art, we used a flow cytometry-based assay to analyze the effects of drugs on parasite growth and parasitemia. Parasite cultures were pulsed (4h) with different concentrations of Art or its derivatives and monitored using SYTO 61, a nuclear dye, through two parasite life cycles (26 and 56 h respectively). Hemoglobin uptake and parasite viability were simultaneously assessed employing parasites that had invaded resealed RBCs containing fluorescein-dextran. We found that drug treatment slows parasite growth and inhibits uptake of hemoglobin, even at sub-lethal concentrations. We also examined whether inhibition of hemoglobin degradation compromises artemisinin activity. The cysteine proteases, falcipain-2 and falcipain-3, play major roles in hemoglobin degradation by intraerythrocytic parasites. Falcipains cleave hemoglobin releasing heme. Inhibition of hemoglobinase activity with the cysteine protease (falcipain) inhibitors, E 64 and ALLN, a calpain inhibitor, significantly decreases artemisinin sensitivity. This finding was substantiated when the falcipain-2 deletion mutant 3D7_ΔFP2 was substantially protected against an artemisinin pulse at the mid-trophozoite stage showing a ~6-fold increase in the IC_{50} value. A fluorescent oxidation reporter, DCF, was used to assess oxidative stress in drug-treated parasites. Art treatment increases the DCF signal, however pre-treatment with the protease inhibitor ALLN completely abrogated the endoperoxide-induced increase in DCF signal. Arrest of hemoglobin digestion by early stage parasites provides a mechanism for surviving short-term artemisinin exposure. Our data strongly suggest that a hemoglobin degradation product (heme or ferrous iron) is needed for the potent antimalarial activity of artemisinin. These insights are important to the design and use of new antimalarials and to the interpretation of emerging data on artemisinin resistance.

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CAPACITY OF *AEDES AEGYPTI* TO VECTOR DENGUE UNDER SMALL AND LARGE DIURNAL TEMPERATURE FLUCTUATIONS

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Few vector competence studies have considered the effect of real-world daily temperature fluctuations on the transmission of vector-borne pathogens. Many investigators have examined the effects of constant temperatures on vector competence, but these are insufficient for understanding how a mosquito's role in transmission is influenced by the variable conditions found in nature. We are investigating the effect of diurnal temperature fluctuations on dengue virus infection and dissemination, and life history traits in the primary mosquito vector *Aedes aegypti*. We use environmentally relevant temperature profiles from Thailand with small (~8°C) and large (~19°C) diurnal temperature ranges around a common mean of 26°C relative to a control temperature regime that is constant 26°C. We are testing the hypothesis that the magnitude of diurnal temperature fluctuations drives seasonal changes in dengue transmission dynamics by influencing components of vectorial capacity; i.e., vector competence and longevity. Our assessment of life

history traits under fluctuating temperature regimes allows us to explore and better understand *Ae. aegypti* population dynamics when they are exposed to various magnitudes of diurnal temperature ranges. Analysis to date shows that large fluctuations in temperature reduce larval survival and slow egg to adult development time by more than 20 hours (X^2 = 71.66, p < 0.01) compared to mosquitoes reared under a constant 26°C. Large daily temperature fluctuations negatively influence the mean number of eggs laid per female (~220 eggs *vs.* 160; $F_{2, 65}$ = 5.44, p < 0.01), eggs per gonotrophic cycle (65 *vs.* 52; $F_{2, 68}$ = 6.01, p < 0.01) and gonotrophic cycles per female (3.3 *vs.* 3.1; $F_{2, 65}$ = 6.93, p < 0.01) after 14 days of feeding on human blood. Results support the notion that fluctuating temperatures affect vectorial capacity and seasonal changes in dengue transmission in Thailand. An improved understanding of realistic temperature fluctuations on *Ae. aegypti*-dengue virus interactions will lead to more effective dengue surveillance and intervention.

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PREDICTION OF DENGUE INCIDENCE USING SEARCH QUERY SURVEILLANCE

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The use of internet search data has been demonstrated to be effective at predicting influenza incidence. This approach may be more successful for dengue which has large variation in annual incidence and a more distinctive clinical presentation and mode of transmission. We gathered freely-available dengue incidence data from Singapore (weekly incidence, 2004-2011) and Bangkok (monthly incidence, 2004-2011). Internet search data for the same period were downloaded from Google Insights for Search. Search terms were chosen to reflect three categories of dengue-related search: nomenclature, signs/symptoms, and treatment. We compared three models to predict incidence: a step-down linear regression, generalized boosted regression, and negative binomial regression. Logistic regression and Support Vector Machine (SVM) models were used to predict a binary outcome defined by whether dengue incidence exceeded a chosen threshold. Incidence prediction models were assessed using r^2 and Pearson correlation between predicted and observed dengue incidence. Logistic and SVM model performance was assessed by the area under the receiver operating characteristic curve. Models were validated using multiple cross-validation techniques. The linear model selected by AIC step-down was found to be superior to other models considered. In Bangkok, the model has an r^2 = 0.943, and a correlation of 0.869 between fitted and observed. In Singapore, the model has an r^2 = 0.948, and a correlation of 0.931. In both Singapore and Bangkok, SVM models outperformed logistic regression in predicting periods of high incidence. The AUC for the SVM models using the 75th percentile cutoff is 0.906 in Singapore and 0.960 in Bangkok. In conclusion, internet search terms predict incidence and periods of large incidence of dengue with high accuracy and may prove useful in areas with underdeveloped surveillance systems. The methods presented here use freely available data and analysis tools and can be readily adapted to other settings.

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ESTIMATES OF THE DEGREE AND LENGTH OF CROSS-PROTECTION BETWEEN DENGUE SEROTYPES FROM TIME SERIES MODELS

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Over sixty years ago, Albert Sabin provided evidence of short-term cross protection between dengue serotypes from human experimental data. Since that time, few other studies have addressed this phenomenon. Several studies have suggested that the inclusion of short-term cross-protection between dengue serotypes is critical to creating transmission models that show behavioral similar to empirical data. However, none of these models have explicitly estimated the duration and strength of cross-protection. Here, we present evidence of short-term cross protection between dengue serotypes using data from a large tertiary hospital in Bangkok (Queen Sirikit National Institute of Children's Health). Our data describes the serotype-specific incidence of hospital-attended dengue from 1973 to 2010. We use a discrete time transmission model to estimate the transmissibility of each dengue serotype as well as the duration and strength of protection provided individuals who have recently been infected with particular dengue serotypes against heterotypic infection and illness. We find evidence of 50-75% protection lasting for 2-3 years. These results are robust to several model formulations. We discuss the dynamic implications of our work and the possible impact on vaccine trials and future vaccine programs.

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THE CHANGING EPIDEMIOLOGY OF DENGUE IN THAILAND: INSIGHTS FROM SEROLOGICAL STUDIES CONDUCTED IN THE SAME LOCATION, 30 YEARS APART

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Dengue fever (DF) and dengue hemorrhagic fever (DHF) have traditionally caused substantial morbidity and mortality among children <15 y of age in Southeast Asia. However, over the past years a significant increase in the mean age of cases has been reported, in spite of a constant number of incident DHF cases. The reasons for this shift are not fully understood. Using data from two age stratified serological surveys conducted among school children in 1980 (n=1009) and 2010 (n=1811) in Rayong, Thailand, we estimated serotype specific forces of infection (FOI), a measure of transmission intensity, and the basic reproductive number (R0) of dengue for the periods 1969-1980 and 1992-2010, respecively. Past exposure to dengue was determined using single dilution neutralization test (SDNT), an assay that differentiates between primary and secondary infection and is serotype specific for those subjects that have been exposed to a single dengue serotype. We found a significant decrease in the FOI, accompanied by a smaller decrease in R0 and critical vaccination fraction. A similar pattern was observed for all four dengue serotypes and when analyzing the data at smaller spatial scales. This is consistent with the idea that the observed age shift might be a consequence of the demographic transitions that Thailand and other SE Asian countries have been undergoing and not of a true decrease in transmission by the vector. We present the evidence for this and other hypotheses. These findings have important implications in the design and implementation of dengue control interventions.

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EXPANSION FACTORS: A KEY STEP IN ESTIMATING DENGUE BURDEN AND COSTS IN SOUTHEAST ASIA

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Dengue is the most common arthropod-borne disease of humans and, unlike most infectious diseases, its incidence is increase. Dengue represents a substantial burden in many tropical and sub-tropical regions, with South-East Asia having the highest dengue incidence. Decisions about the implementation of existing and new technologies require information about the disease burden. The most difficult step is estimating disease incidence accurately. The total incidence of symptomatic dengue illness is not fully captured by surveillance systems, which usually under report the number of cases. The total number of dengue cases may be estimated using an expansion factor (EF), the number by which reported cases need to be multiplied to obtain the true number. This study is using EFs to project dengue cases and costs throughout SE Asia by year. We conducted a systematic literature review (1995-2011) and identified 9 published papers reporting original, empirically derived EFs or the necessary data from SE-Asia. EFs are based on: total cases/hospitalized case (H), total cases/diagnosed dengue (D), or total cases/reported case (R). Two EFs are based on a study of children cohorts in Kamphaeng Phet, Thailand (4.8H,3.4H), another looks at children cohorts in Kamphaeng Phet and Ratchaburi (8.4R); two are from Bandung, Indonesia--based on dengue hemorrhagic fever surveillance in 4 major hospitals (4.3R) and surveillance of a cohort of adults (2.3H); two are from Viet Nam--children cohorts in Long Xuxen (5.8R) and patients at community health posts at Binh Thuan (6.2D); and finally, two are from active community-based surveillance of children in Kampong Cham, Cambodia (7H, 9.3R). Despite SE Asia's long standing surveillance systems, these studies documented considerable underreporting. EFs in SE Asia varied by dengue definition, age group, urban/rural, geography, etc., and ranged from 2.3H (adults, Vietnam) to 9.3R (children, Cambodia). In conclusion, studies that make no adjustment for underreporting would seriously understate the burden and cost of dengue in SE Asia.

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DENGUE DYNAMICS IN THE 2009 OUTBREAK IN ORAN, ARGENTINA: IMPLICATIONS FOR MONITORING AND CONTROL

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After more than eighty years Dengue reemerged in Argentina in 1997. Since then, the largest epidemic in terms of geographical extent, magnitude and mortality, was recorded in 2009. In this work we analyzed the space-time dynamic of the Dengue epidemic in Oran, Salta province, one of the main epicenters of the outbreak. We also studied its correlation with demographic, socioeconomic and entomological factors. The city of San Ramon de la Nueva Oran is located in one of the main route of introduction of Dengue to northwest Argentina. Cases were diagnosed by UM-ELISA and MAC-ELISA (IgM) between January and June 2009. Demographic and socioeconomic data by neighborhood were obtained from the Provincial Statistics Direction. Diagnosis date and place of residence of patients were entered on a Geographic Information System using vector format cartography and Gauss Kruger coordinates into ArcGIS 9.3 software. We applied a space-time scan statistic under Poisson model considering city neighborhoods as the spatial unit and day as the temporal unit. Spearman correlation was used to study associations between socioeconomic variables and Dengue incidence. Larval house (LH) and Breteau (B) indices of *Aedes aegypti* space-time distribution was smoothed by kernel density. The epidemic started from an imported case from Bolivia which generated two seminal clusters on February 26 and 27, in the northeast and the south of the city with risk ratios of 32.9 and 36.4 respectively ($p < 0.001$). Following cases spread around the city without significant space-temporal clustering. No statistically significant association between socioeconomic variables and dengue incidence by neighborhood was found but positive correlation between population size and the number of cases ($p<0.05$) were detected. Larval indices show maximum values for the month of January (B=21.96; HL=8.39) with a gradual decrease until June. The lack of correlation between socioeconomic variables and incidence show that in this case socioeconomic conditions are not risk factors for Dengue transmission.

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RE-EMERGENCE OF DENGUE VIRUS SEROTYPE 3 IN PUERTO RICO: CHARACTERIZATION OF A DISTINCTIVE EXPANSION PATTERN

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Dengue virus (DENV) exists as four serotypes, each containing multiple genetically distinct genotypes. Colonization drives divergence of distinct lineages, generally grouped by region of isolation. Following a 21 year absence of DENV-3, the Indian Subcontinent strain emerged in Puerto Rico in 1998. The subsequent, progressive expansion of DENV-3 in Puerto Rico correlates with the displacement of the other serotypes and is representative of what occurred in the rest of the Americas. We sequenced complete genomes of 92 DENV-3 isolates obtained from clinical cases to characterize genetic diversity, phylogeography, and molecular evolution throughout 10 years of continued sampling in Puerto Rico (1998-2007). Genetic lineages were then associated with temporal and geographical data. This analysis shows that five distinct lineages emerged almost simultaneously and evolved independently. Two of these lineages are associated to strains from the Caribbean basin and were transmitted on the island for short periods. The other three lineages are formed of autochthonous virus of foreign origin, of which two successfully accomplished long-term expansions. Temporal clustering associated to specific geographical regions was found within these three autochthonous lineages. We found evidence of sustained microevolution within the clusters and fostering of fast virus migration from these clusters to the rest of the island. These local lineages experienced a steep increase in genetic diversity during the first 5 years of expansion to then stabilize during the years of full dominance of this serotype. This is the first extensive study of DENV-3 emergence and evolution in the region. Our findings unveil a high genetic diversity and co-transmission of DENV lineages coupled with a complex dissemination pattern that is different to the evolution of every other serotype on the island. Research to further define the biological and epidemiological determinants of these transmission patterns may aid our efforts to prevent the spread and re-emergence of dengue in endemic areas.

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IMMUNOGENICITY AND EFFICACY OF A SAND FLY-BASED *LEISHMANIA* TRANSMISSION-BLOCKING VACCINE FOR CANINES

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Leishmania infantum (= *chagasi*) is the causative agent of zoonotic visceral leishmaniasis; a fatal parasitic disease if left untreated. *Leishmania*

parasites undergo two developmental cycles and one of these cycles takes place within the midgut of a sand fly. The molecular interaction between the sand fly midgut and *Leishmania* parasites is poorly understood. By performing transcriptomic analyses we identified several molecules that potentially interact with the parasites in the midgut of *Lutzomyia longipalpis*, the natural vector of *L. infantum* in Latin America. Among those, we sought to select molecules that could interfere with parasite development inside the sand fly midgut and ultimately prevent transmission of the parasite to a mammalian host. Molecules abundantly transcribed during blood meal digestion were selected as ideal targets for transmission-blocking vaccines and antibodies were generated against these molecules via DNA vaccination. Sand flies were infected by an artificial blood meal containing *L. infantum* promastigotes and naive or target-immunized mouse sera. One particular *L. longipalpis* midgut molecule, LuloPer1, generated antibody that reduced the parasite load within the sand fly and resulted in a significant decrease in the mean number of parasites present during the infectious stage of the sand fly by 71%. Additionally, feeding anti-LuloPer1 antibody decreased survival by 27% six days after the blood meal. Canines are the principal reservoir for *L. infantum* in Latin America; thus, we are currently vaccinating dogs with LuloPer1 to assess its immunogenicity and efficacy in canines. For this purpose, recombinant LuloPer1 was expressed in a eukaryotic system and purified. Encouragingly, preliminary data show that vaccination of uninfected and infected asymptomatic dogs elicits a specific antibody response to LuloPer1. The identification of a molecule that can reduce or abrogate the transmission of *L. infantum* by *L. longipalpis* has implications for public health, blocking the spread of *Leishmania* between dogs and from dogs to humans.

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CAUDAL REGULATES THE TRIPARTITE INTERACTIONS BETWEEN THE INNATE IMMUNE SYSTEM, THE MICROBIOTA AND THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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Anopheles gambiae, the major vector for the human malaria parasite *Plasmodium falciparum* in sub-Saharan Africa, uses its innate immune system to defend against *Plasmodium,* mainly via the Toll and Imd (Immune Deficiency) signaling pathways. Interestingly, these immune pathways are also activated by the microbiota present in the mosquito midgut, which is the primary site for *Plasmodium* invasion and development (Dong *et al.,* 2006 and 2009).*Caudal* was first identified in *Drosophila* as a developmental transcription factor as well as a negative regulator of the Imd pathway -mediated activation of the Relish transcription factor, as reported previously. We have shown through RNAi-based silencing assays that depletion of the *An. gambiae Caudal* results in a significant reduction of the midgut microbiota as well as a change of its species composition. Additionally, antimicrobial peptides (AMPs) are significantly upregulated upon silencing of *Caudal*. We also present studies on *Caudal*'s role in regulating the midgut microbial load and composition in field-derived *Anopheles arabiensis* mosquitoes, a key vector of malaria in southern Zambia. In these studies, the silencing of *Caudal*-silenced mosquitoes had approximately two-fold less bacteria compared to wildtype mosquitoes. Interestingly, *Caudal* is also a highly potent regulator of vector competence for *P. falciparum* while its implication in the defense against the rodent parasite *P. berghei* was weak. Our previous studies have also shown that the Imd pathway more efficiently defends against *P. falciparum* than *P. berghei* as reported previously. These findings suggest that the *An. gambiae Caudal* can influence the finely tuned tripartite interactions between the innate immune system, the midgut microbiota, and the *Plasmodium* parasite as a factor of the Imd pathway. We are currently conducting comprehensive whole-genome microarray gene expression studies to better understand *Caudal's* relationship to the Imd and Toll pathways and to identify potent anti-*Plasmodium* effectors that are transcriptionally controlled by this immune response regulator.

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REGULATION OF ANTI-*PLASMODIUM* IMMUNITY BY THE TRANSCRIPTION FACTOR LL3 IN *ANOPHELES GAMBIAE*

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Mosquitoes of the genus *Anopheles* serve as the obligate vectors of the malaria parasite *Plasmodium*. During its development within the mosquito host, several factors and developmental bottlenecks limit parasite success, including the mosquito innate immune response. Recently, we have identified an ortholog of the vertebrate transcription factor LITAF in the *Anopheles gambiae* genome named LITAF-like 3 (LL3). In response to midgut invasion by mouse and human malaria parasites, LL3 is up-regulated in the mosquito midgut epithelium. Oocyst numbers are significantly increased upon the RNAi-mediated knockdown of LL3, implicating its involvement in limiting *Plasmodium* parasite success. Upon dsRNA knockdown of LL3, the mRNA abundance of SRPN6 (an inhibitor of *Plasmodium* development) is significantly decreased in the mosquito midgut. In addition, electrophoretic mobility shift assays demonstrate that recombinant LL3 protein binds to regulatory regions within the SRPN6 promoter, suggesting that LL3 directly regulates SRPN6. Further identification of the downstream targets of LL3 was conducted by microarray analysis, resulting in the differential expression of 747 probes. Current experiments aim to identify the function of a subset of these genes affected by the knockdown of LL3 and to elucidate the mechanism by which LL3 confers anti-*Plasmodium* defenses in the mosquito midgut.

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DISSECTING THE PIRNA PATHWAY'S ROLE IN TRANSPOSON CONTROL IN THE IMPORTANT DISEASE VECTOR *AEDES AEGYPTI*

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The most recent class of small RNAs to be discovered is the Piwi interacting piRNA. PiRNAs were first discovered and reported in mice. PiRNA biogenesis is distinct from the siRNA and microRNA biogenesis in that it is involved in a dicer-independent pathway. PiRNAs are 24-30nt small RNAs that bind Piwi proteins. The Piwi class proteins were first discovered over ten years ago in drosophila mutants and are members of the Argonaute clade. Currently piRNAs are believed to be involved in transposon and endogenous retrovirus control in both the germline and somatic cells, as reported previously. The biogenesis pathway of piRNAs is the least understood of the current three small RNA pathways. The ping-pong model of piRNA production suggests that Argonaute proteins work together in an autoamplification loop and is currently the strongest hypothesis being considered, as reported previously. The role piRNAs play in transposon control is only beginning to be understood. The important proteins involved in the pathway have been identified but the details in how they function together to create a targeted response are still not fully worked out. Our research investigates the function, expression and targets of the piRNAs associated with the PIWI protein in *Aedes aegypti*. To date no investigations into the piRNA pathway have been performed in mosquito systems. Here we aim to determine the tissues and developmental stages in which the putative Piwi proteins are expressed in the medically significant *Aedes aegypti*. We have characterized the piRNAs that are bound to the Piwi protein via co-immunoprecipitation *in vivo*. The isolated piRNAs were analyzed bioinformatically and mapped to the *Aedes aegypti* genome to determine the targets of these small RNAs. The information provided in this study provides valuable insight into how

the Piwi proteins are functioning in mosquitoes. This knowledge may in turn help us understand transposon control thus enabling us to develop techniques to circumvent transposon silencing and boost transformation efficiency of mosquitoes

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BRUMMER LIPASE AND ADIPOKINETIC HORMONE LIPOLYTIC PATHWAYS ARE REQUIRED TO GENERATE LIPIDS NECESSARY FOR TSETSE MILK PRODUCTION

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Tsetse females reproduce by obligate viviparity, generating a single larva per gonotrophic cycle, and larval nourishment is derived from milk secretions provided by the mother. Optimum nutrition during pregnancy requires mobilization of large quantities of lipids for production of milk during intrauterine larval development. We investigated the role two lipolytic pathways (Brummer lipase (Bmm) and adipokinetic hormone (AKH)/AKH receptor pathways) on providing lipids for milk production. Two putative adipokinetic hormone (AKH) coding genes, (*akh* and *hrth*), *akhr* and *bmm* were identified from *Glossina morsitans morsitans*. Expression of *akh*, *hrth* and *akhr* increase at the end of oogenesis during the first gonotrophic cycle, then decrease and stabilize throughout larval development. Levels of *bmm* increase during the early progeny development and decline at parturition. Knockdown of *bmm* (*bmm*$^-$) and *akhr* (*akhr*) was accomplished utilizing siRNA injection. Suppression of one lipolytic pathway results in an increase in transcription of the other. Starvation-based experiments on females revealed thay *bmm*- and *akhr*- flies had prolonged survival. Simultaneous reduction of both genes extends survival by an additional 20%. Flies with the *akhr*/*bmm*$^-$ knockdown have higher lipid contents upon death, indicating the inability to completely utilize stored lipid reserves. Oocyte development was impaired by knockdown of *bmm* and impairment was even more dramatic in *akhr*/*bmm*$^-$ flies. Flies with *akhr*$^-$ and *bmm*$^-$ knockdown have 20 and 50% reduction in fecundity, respectively, and *akhr*$^-$/*bmm*$^-$ flies have an 80% decrease. Omission of one bloodmeal (short period of starvation) for *akhr*/*bmm*$^-$ flies leads to almost complete suppression of reproduction. The reduced level of fecundity is likely due to the inability of tsetse to utilize lipid reserves as the *akhr*/*bmm*$^-$ phenotype leads to increased lipid accumulation and retention, particularly during pregnancy. These studies show that Bmm and AKH/AKHR pathways are critical to producing lipids necessary for milk production during tsetse fly pregnancy.

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MEASUREMENT OF THE SCUTAL INDEX AND DISPARATE ACQUISITION OF *BORRELIA BURGDORFERI* GENOTYPES IN LARVAL *IXODES SCAPULARIS* TICKS

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Borrelia burgdorferi, the causative agent of Lyme borreliosis in the United States, is comprised of many genotypes, which are associated with varying degrees of dissemination in host species. These genotypic strains are known to infect *Ixodes scapularis*, the primary vector, disparately. Field studies often examine larval-stage ticks removed during their host feeding periods as an indicator of infection in the host. Characterization of these various genotypes during larval feeding has yet to be done, adding a source of uncertainty in determining host infection. In addition, feeding duration needs to be correlated with pathogen acquisition in order to associate various time points during feeding with the probability of a tick becoming infected. Here we quantitatively describe how *I. scapularis* larvae obtain two different genotypes of *B. burgdorferi* during 12 hour intervals over the course of the feeding period. Uninfected larvae were placed on *Peromyscus leucopus* previously infected with either a highly-disseminating or low-disseminating strain, Bb206 or Bb348. Larvae were removed every 12 hours for 72 hours. We measured the scutal index as an indicator of feeding duration and ticks were assayed using a quantitative PCR protocol. The scutal index revealed a direct relationship between scutal index measurement and length of feeding. Results contrast significantly between the two strains of Borrelia. Ticks infected with Bb206 had increased quantities of Borrelia per tick and produced more infected ticks at each time point of feeding as compared to Bb348, but was only significant at hours 36, 42, and 72. Ticks that fed on Bb206-infected mice acquired infection during the first 12 hours of feeding, whereas ticks feeding on Bb348- infected mice required more time to become infected. In summary, our findings further describe the intricate infection characteristics of *B. burgdorferi* and will enable researches to make better estimations of infection prevalence in mammals based on the infection status of removed larval ticks.

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RHODNIUS PROLIXUS GENOMICS AND TRANSCRIPTOMICS: IDENTIFICATION AND ANNOTATION OF GENES LINKED TO THE Y CHROMOSOME AND GENES RELATED TO SEX DETERMINATION

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Due to the abundance of repetitive DNA, the sequencing and assembling of genomic heterochromatic regions is problematic. The Y chromosome is heterochromatic in most species, hindering the identification of Y-linked sequences in many genome projects. Our laboratory has developed a variety of computational and experimental methods to overcome such difficulties. These methods have allowed a series of comparative studies on the gene content of the *Drosophila* Y chromosome and we have shown recently, that the *Drosophila* Y chromosome may have a non-canonical evolutionary history. Therefore, the study of the Y chromosome of other insects may reveal new aspects of this chromosome, helping to better understand male fertility and sex differentiation systems in other arthropods. The kissing bug *Rhodnius prolixus* is the vector of the parasite *Trypanosoma cruzi* (the etiological agent of Chagas' Disease, an important neglected disease) and its genome project is in progress. By using the expertise obtained during the 12 *Drosophila* genome project, we have proposed to identify and annotate the genes linked to the Y chromosome of *R. prolixus*. We have already identified 3.8 Mbp of Y-linked sequences. We are also using transcriptome information to find genes of interest. The genomic and transcriptomic analysis revealed hundreds of potential Y-linked genes, and we are working on the full annotation of 20 of these genes. We also uncovered most of the genes related to sex determination in arthropods (e.g. *sex lethal, doublesex* and *fruitless*), suggesting that Hemiptera insects may have a sex determination system similar to Diptera. Our preliminary results have revealed that separate sequencing of male and female genomic DNA may turn out to be a powerful method for finding sequences of the Y chromosome. The combination of methods proposed here may help us to better understand the mechanisms involved not only in the origin and evolution of sex chromosomes, but also the mechanisms involved in sex determination and male fertility of non-dipteran arthropod vectors.

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ENTAMOEBA MOSHOKOVSKII IS PATHOGENIC AND CAUSES INTESTINAL SYMPTOMS

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Entamoeba moshkovskii is prevalent in the developing countries and morphologically indistinguishable from pathogenic *E. histolytica* and non-pathogenic *E. dispar*. As *E. moshkovskii* has been considered to be a non-pathogenic or a free living amoeba, there are few studies to elucidate the pathogenicity of it. Thus, we carried out the study to clarify the pathogenicity of *E. moshkovskii*. To examine the pathogenicity of *E. moshkovskii*, animal model of intestinal amoebiasis was utilized. Trophozoites of *E. moshkovskii, E. histolytica* or *E. dispar* were challenged to mice and infection rate was examined. In successfully infected mice, intestinal symptoms, weight loss and time course of infection were observed. Then the prevalence of each *Entamoeba* spp. in diarrheal episode was examined in Bangladesh and several cases that were positive only for *E. moshkovskii* but negative for other conceivable diarrhea-causative microbes were found. *E. moshkovskii* settled in CBA/J, C3H/HeN and C3H/HeJ mice, but not in C57BL/6J and BALB/c mice similar to pathogenic *E. histolytica*, while non-pathogenic *E. dispar* could not establish the infection in mice. *E. moshkovskii* induced intestinal symptoms and weight loss in mice. In Mirpur, Dhaka, Bangladesh, *E. moshkovskii* was identified in 42 diarrheal episodes (2.95%) out of 1426 diarrheal episodes in 385 children, while *E. histolytica* was in 66 (4.63%) and *E. dispar* was in 5 (0.35%), indicating the association of diarrhea with pathogenic *E. histolytica* and *E. moshkovskii*. Six episodes were confirmed to be solely associated with *E. moshkovskii*, without detection of any conceivable diarrhea-causative microbes. *E. moshkovskii* was found to be pathogenic to mice and to well associate with diarrheal episode in Bangladeshi children. Therefore it is important to re-estimate the pathogenicity of *E. moshkovskii*.

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COMMON PATHWAYS FOR THE RECEPTOR-MEDIATED INGESTION OF *ESCHERICHIA COLI* BACTERIA AND LDL CHOLESTEROL BY *ENTAMOEBA HISTOLYTICA* REGULATED BY TRANSMEMBRANE KINASE (TMK) 39

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The single-celled parasite *Entamoeba histolytica* is an enteric pathogen that ingests bacteria and host cells as it causes intestinal disease. The ligand/receptor interactions that allow *E. histolytica* to phagocytose such extracellular targets are not well understood. We hypothesized that trophozoites of this parasite might accomplish ingestion through the utilization of scavenger receptor-like mechanisms. Here we show that acetylated and oxidized forms of LDL cholesterol (AcLDL and OxLDL) were phagocytosed by ameba via receptor-mediated mechanisms, whereas pinocytosis of dextran was not. AcLDL competitively inhibited the ingestion of *E. coli* bacteria, but not erythrocytes and Jurkat T lymphocytes, by 31% (SE±1.34) (P<.005), suggesting a common phagocytic pathway. Inducible expression of a truncated dominant-negative version of *E. histolytica* transmembrane kinase 39 (TMK39) inhibited ingestion of *E. coli* by 55%±2.99 (P<.005). We concluded that two pathogenic processes, ingestion of AcLDL and of *E. coli,* shared common pathways and regulation.

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EXPRESSION AND PURIFICATION OF RECOMBINANT LECA PEPTIDE AS A CANDIDATE FOR AN AMEBIC COLITIS VACCINE

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Entamoeba histolytica causes amebic colitis and liver abscess. It is a major enteric pathogen in developing countries and for travelers returning from endemic areas. The interaction of *E. histolytica* with the intestine occurs through the binding of the trophozoite stage via a Gal/GalNAc lectin comprised of disulfide linked heavy (180 kDa) and light chains (35 kDa) and noncovalent binding with an intermediate subunit (150 kDa). Our efforts to develop a vaccine against this pathogen have focused on an internal 578 amino acid peptide, LecA, within the cysteine-rich region of the heavy chain subunit because (i) it is a major target of the cell mediated and humoral immune response of immune individuals and (ii) vaccination with LecA provides protection in animal models. We developed a process for obtaining >95% homogeneous LecA. The lecA gene sequence was optimized for expression in Escherichia coli, and LecA was expressed in host strain HMS174 in the vector pJ express 401 containing a Kanr gene and a T5 promoter. More than 80% of the peptide was expressed in inclusion bodies (IB). The process consisted of three stages: (i) cell lysis, collection and washing of IB; (ii) solubilization and refolding of LecA; and (iii) Superdex 200 gel filtration. SDS-PAGE demonstrated a major peptide (70 kDa) identified as LecA by N-terminal sequencing and tryptic digest analysis. The LecA band and minor bands reacted with monoclonal antibodies against LecA. LecA had an apparent MW of 70,000 by SDS-PAGE compared to 158,000 by gel filtration. Endotoxin levels were 0.20 EU/µg, and DNA and RNA levels were <200 ng/mg and <20 ng/mg, respectively. The purified peptide exhibited higher residual immunoreactivity than his-tagged LecA, which is protective in a murine model of amebic colitis, indicating that protective epitopes were conserved. The procedure is scalable to cGMP and yields 20 mg per liter shaking flask culture. Our procedure yields sufficient amounts of highly purified LecA for future studies on stability, immunogenicity, and protection studies.

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VALIDATING MULTIPLEX REAL-TIME PCR FOR THE DETECTION OF INTESTINAL PARASITES IN THE CLINICAL LABORATORY

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A molecular diagnostic strategy was evaluated for the routine diagnosis of intestinal parasites in stool specimens. The possibility of high throughput multiplex detection of stool intestinal parasites may ultimately result in rapid and sensitive testing. Unpreserved stool specimens were collected from symptomatic patients samples in Toronto, Ontario. Stool samples were analyzed on a routine basis by microscopic examination for ova and parasites. Both positive and negative stool samples were banked in a stool biorepository by preparing five aliquots of each specimen in a 1.5 ml screw capped vials and stored at -20°C for batched molecular testing. Multiplex real-time polymerase chain reaction (RT-PCR) analysis was designed to detect *Entamoeba histolytica*, *E, dispar*, *Giardia lamblia*, *Cryptosporidium* (*C. parvum / C. hominis*), *Dientamoeba fragilis, Cyclospora cayetanensis, Strongyloides stercoralis, Necator americanus*, and *Ascaris lumbricoides* using a single or two multiplex panels in 96 well plates. In a pilot run the RT-PCR was performed on 50 stool samples

which included 10 negative and 40 positive stool samples by routine microscopy. The 40 positive stool samples included *E. histolytica* (n=10), *Giardia lamblia* (n=4), *Cryptosporidium* (n=3), *Dientamoeba fragilis* (n=10), *C. cayetanensis* (n=10), *Strongyloides stercoralis* (n=2), and *Microsporidium* (n=1) by microscopy. The RT-PCR result was concordanct with all microscopy positive stool samples. In addition, RT-PCR also picked a mixed infection (*Strongyloides stercoralis* and *Isospora belli* and *Necator americanus*) in one stool sample which was exclusively positive for *S. stercoralis* by microscopy. Interestingly, the RT-PCR was positive in 2 out of 10 stool samples negative by microscopy: *Schistosoma mansoni* and *Enterocytozoon bieneusi*. In summary, we have developed a multiplex RT-PCR panel to simultaneously detect eleven stool intestinal parasites. The pilot study conducted demonstrated excellent concordance with microscopy. Furthermore, several specimens negative by microscopy were positive by RT-PCR for significant stool pathogens. Superior sensitivity and throughput of multiplex RT-PCR methods in comparison to routine microscopy suggest that increased detection rates and improved turn around times may be possible for certain stool parasites.

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EFFECTS OF HABITAT DISTURBANCE ON HOST COMMUNITY STRUCTURE AND PATHOGEN PREVALANCE

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Infectious diseases are strong forces acting on natural populations and considered an important component in the structure of ecosystems. Disease dynamics can rarely be explained by examining one component of a complex natural system. Habitat quality, host community, host health status, and pathogen interactions are interconnected in complex and dynamic ways. To understand disease dynamics in natural settings, the complex interplay among habitat disturbance, host community structure, and pathogen interactions with both the host and any co-infecting pathogen must be understood. This study examines the small mammal community in western Uganda and the pathogens infecting these animals. We collected 348 small mammals from habitats experiencing varied levels of habitat disturbance in and around Kibale National Park, western Uganda. Each animal was identified to species level and screened for the presence of ectoparasites, protozoans, and viruses. Our results suggest that increases in habitat disturbance are linked, to a point, with an increase in diversity of small mammals and prevalence of their pathogens. We aim to more accurately capture the true complexity and dynamic nature of pathogen-host ecology by investigating the interactions between multiple sympatric pathogen-host systems in areas experiencing varied levels of disturbance.

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MONITORING TRANSMISSION OF *WUCHERERIA BANCROFTI* AMONG A SENTINEL COHORT OF PAPUA NEW GUINEAN CHILDREN USING A COMBINATION OF DIAGNOSTIC ASSAYS

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Monitoring of existing national lymphatic filariasis (LF) elimination programs is based on the WHO recommended immunochromatographic test (ICT) that detects adult *Wuchereria bancrofti* (Wb) antigen in blood. Though convenient for identifying areas eligible to start mass drug administration (MDA), the ICT shows variable efficacy following longstanding MDA programs. To evaluate whether newer serological measures of assessments of Wb infection/exposure would be more useful than the ICT test, a sentinel population of 1-10 year old children was studied to assess the best approach. Serum samples were obtained from children born in communities that had completed 5 rounds of annual MDA ten years previously. These samples were evaluated with an L3 stage-specific WB-123 LIPS assay and compared to LF monitoring tools including microscopy for microfilaria (MF), PCR, circulating antigen tests (ICT and TropBio™), and an alternative antibody test (Bm14). Among the 422 children studied, 9 were MF positive. With regard to the 9 MFpositive children, tests for antigen and antibody to BM14 were 100% concordant. One and two MF positive children were negative by WB-123 and PCR, respectively. Ability of an assay to detect suspected LF transmission (i.e., highest sensitivity) was evaluated as the percent of 413 MF negative children who tested positive in each alternative assay. Increased sensitivity over microscopy for MF was 3% for PCR, 5% and 9% for antigen (Binax and Og4C3, respectively), and 11% and 26% for antibody (Wb-123 antibody and BM14, respectively). Test positivity among MF negative children may represent pre-patent, single-worm, non-fecund infections, or non-infective exposure to Wb. Results show varying outcomes observed when targeting different parasite and host bio-markers of Wb infection. LF elimination programs must consider the strengths and limitations of diagnostic strategies in effective monitoring and evaluation of LF Elimination Programs.

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ANTIBODY TO L3 ANTIGEN (WB123) AS A MARKER OF BANCROFTIAN FILARIASIS TRANSMISSION IN THE SOUTH PACIFIC

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Antibody (Ab) to the *Wuchereria bancrofti* (Wb) infective larval (L3) antigen Wb123, detected by a Luciferase Immunopreciptation System (LIPS) assay, has been shown to be a species-specific, early marker of infection with Wb developed for its potential use as a surveillance tool following transmission interruption. To examine its usefulness in a single filarial-endemic island (population ~600) assessed at two time points with markedly different levels of transmission, Ab to Wb123 was measured in sera collected from subjects in Mauke, Cook Islands in 1975 (n=369; no previous treatment) and 1992 (n=553; 5 years after a one time island-wide treatment with diethylcarbamazine [DEC]). Between the two time points, Wb transmission had decreased dramatically as evidenced by reduced prevalence of microfilariae (31% vs 5%) and circulating Ag (CAg, 49% vs 16%). Age specific prevalence analysis showed an even more dramatic reduction in Wb123 Ab positivity from 54% (25/46) in 1975 to 8% (3/38) in 1992 in children 1-5 years ($p<0.0001$), reflecting the single-dose DEC treatment five years earlier. By 1992, Wb123 Ab prevalence in children 6-10 years had fallen from 75% (42/56) in 1975 to 42% (33/79) reflecting a lower cumulative transmission potential. In the whole population, Wb123 seropositivity decreased from 86% to 61% between 1975 and 1992. In CAg+ subjects the levels of Wb123 Ab were indistinguishable between the 2 time points (geometric mean [GM]=232,067 units [1975] vs 210,115 [1992]) but differed in those who were CAg- (GM=33432 [1975] vs 11,095 [1992]; $p<0.0001$). In paired sample analysis, individuals who were CAg+ in 1975 but became CAg- in 1992 had significantly lower Ab levels in 1992 ($p<0.0001$), with 9/40 (23%) becoming seronegative for Wb123. The clear relationship between reduction in Wb123 Ab prevalence and the reduction of transmission, seen most clearly in young children, strongly advocates for the continuing assessment and rapid development of Wb123 as a surveillance tool to detect potential transmission of bancroftian filariasis in treated endemic areas.

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USE OF ENHANCED SURVEILLANCE METHODS FOR DETECTING LOW-LEVEL PERSISTENCE OF LYMPHATIC FILARIASIS FOLLOWING CESSATION OF MASS DRUG ADMINISTRATION (MDA)

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The Sri Lanka Anti-Filariasis Campaign (AFC) provided MDA to 10 million people in 8 endemic districts between 2002 and 2006. All districts satisfied WHO criteria for filariasis (LF) elimination in 2008, but surveys showed low-level persistence of microfilaremia (Mf) in some sentinel sites. This project is using enhanced surveillance tools to detect persistent LF. Methods include community surveys to detect Mf and filarial antigenemia (ICT), school surveys for ICT and anti-filarial antibodies (Bm14 ELISA) in children 6-8 years of age, and mosquito surveys to detect filarial DNA in Culex mosquitoes collected by gravid traps (molecular xenomonitoring, MX). Criteria for LF elimination were predefined to be <0.5% for Mf (community), <2% for ICT (community), <2% for antibody in children, and <0.25% for parasite DNA in mosquitoes. The project will test two sentinel sites with populations ranging from 10,000 to 35,000 in each of the 8 formerly endemic districts. We now report results from study site A in Gampaha district and study sites B and C in Colombo district. All laboratory testing was conducted by AFC personnel. Community Mf rates were very low in all three areas (< 0.5%). Community ICT rates were 3.6, 0, and 0.5% in areas A, B, and C. ICT rates in children were 1.6, 0, 0% in areas A, B, and C, and antibody rates in children were 2% in areas B and C. Filarial DNA rates in mosquitoes in areas A, B, and C were 0.75, 0.09, and 0.5%. Thus all three study areas had low-level persistence of filariasis markers several years after suspension of MDA. Areas A and C failed to meet our criteria for LF elimination; follow-up testing will be needed in these areas. WHO recommends using child ICT survey results as the primary indicator for decisions to stop MDA and for post-MDA surveillance. However, antibody testing of children and MX appear to be more sensitive tools for detecting low-level persistence or resurgence of filariasis in communities. We recommend use of these tools for special surveys in suspected hot spots to complement systematic ICT surveys currently recommended by WHO.

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LONGITUDINAL EVALUATION OF ANTIFILARIAL SEROLOGICAL RESPONSES IN A COHORT OF HAITIAN CHILDREN

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Antifilarial antibody testing has been established as a sensitive and specific method of diagnosing lymphatic filariasis. However, the development of serological responses to specific filarial antigens and their relationship to acquisition of infection is poorly understood. In order to evaluate whether the detection of antifilarial antibodies precedes that of microfilaremia and antigenemia, we compared the antibody responses of serum samples collected between 1990 and 1999 from a cohort of 143 Haitian children followed longitudinally. Antigen status was determined using the Og4C3 ELISA and the presence of microfilaremia was detected using microscopy. Antibody responses to Wb123, a *Wuchereria bancrofti* L3 antigen, were measured using a Luciferase Immunoprecipitation System (LIPS) assay. Antibody responses to Bm14 and Bm33, *Brugia malayi* antigens, and to a major surface protein (WSP) from *Wolbachia* were analyzed using a multiplex bead assay. The median month of positivity (MM) to all parameters was determined. Over follow-up, 81 (57%) of the children became Ag+ (MM= 48), and 43 (30%) developed microfilaremia (MM= 70). Detectable antibody responses to Bm14 (MM=42), Bm33 (MM=33), Wb123 (MM=45), and WSP (MM=58) developed in 95%, 99%, 90%, and 22% of children, respectively. Peak incidence of antibody was 3, 2.5, and 2 years for Wb123, Bm14, and Bm33, respectively. Both Bm14 and Wb123 antibody prevalence were significantly greater (P<0.05) among children who became Ag+ than among Ag- children. Antifilarial antibody responses can serve as an important epidemiological indicator in a sentinel population of young children. Understanding the timing of the development of antibody responses will help to establish a framework for using antibody testing for surveillance of lymphatic filariasis in the effort to eliminate the disease.

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ARE FIVE ANNUAL ROUNDS OF MASS DRUG ADMINISTRATION (MDA) ENOUGH TO ELIMINATE LYMPHATIC FILARIASIS (LF) IN EGYPT?

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Egypt (with an estimated 2.7 million population at risk of LF) initiated one of the first national LF elimination programs (EPELF) based on mass drug administration (MDA) with DEC and albendazole in 2000. The program used villages as implementation units (IUs) and included IUs with baseline infection rates ≥1% mf. The program maintained high coverage rates (≥80% by independent surveys). MDA was discontinued in 149 IUs (92.5% of the total) that met WHO stopping criteria after 5 rounds. As these criteria have not been evaluated extensively, the present study was designed to test the WHO hypothesis that 5 MDA rounds with good coverage would eliminate LF. We selected 5 villages with the highest pre MDA mf prevalence (4 - 11%) to be our evaluation units (EU) in this study that was carried out in 2010 to find out if LF resurgence would happen in 6 years after stopping the MDA. We examined ~400 adults and all first primary school kids (age 6 - 7 years) in each village for antigenemia using ICT cards (total of 2095 adults and 1026 children). All were found to be antigen-negative. We also carried out an outdoor mosquito survey using 40 Gravid Mosquito Traps (GMT) distributed at the periphery of each village. 50 mosquitoes (the main LF vector in Egypt being Culex pipiens) were collected from each trap. Half were analysed by PCR in the Central Lab of the Egyptian Ministry of health while the other half were shipped to the US for quality control. All specimens were PCR-negative. These two sets of findings imply that no resurgence of LF occurred in Egypt in the 6 years after stopping MDA.

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LONG LASTING INSECTICIDE TREATED NETS COMPLEMENT MASS DRUG ADMINISTRATION TO ACCOMPLISH A SUSTAINED REDUCTION IN LYMPHATIC FILARIASIS TRANSMISSION

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The principle strategy of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) employs annual mass drug administration (MDA) for 4-6 years to break LF transmission. Despite successes, GPELF has yet to overcome challenges of attaining high drug coverages and sustaining programs for multiple consecutive years. Also, elimination thresholds are unknown in most areas. These difficulties have led to concern that a single global strategy of elimination may not be resilient, and specifically, that lack of vector control may hinder the progress of LF elimination. There are many areas where *Anopheles* spp. transmit both LF and malaria parasites, providing the opportunity for malaria control efforts to benefit the GPELF. Currently, the most widely implemented vector control intervention in malaria campaigns is the distribution of long lasting insecticide treated nets (LLINs). However, only one study has demonstrated the positive impact of LLINs on reducing LF transmission by anophelines, and there is no data that quantifies how LLINs will complement MDA. Distribution of LLINs following MDA may increase the probability of a sustained reduction in LF transmission. To test this hypothesis, we collected mosquitoes before and after LLIN distribution (n=21,642) in an area of Papua New Guinea that had previously received MDA. Mosquitoes were examined for infection by *Wuchereria bancrofti*, and a subset was analyzed for *W. bancrofti* DNA. Shortly after LLINs were distributed, surveys indicated that 83% of study participants slept under an LLIN (n=2459). Entomological indices of transmission in 1998 (after MDA) and in 2008 (prior to LLIN) were similar. The *An. punctulatus* man biting rate was significantly reduced post LLIN. Significantly fewer mosquitoes were infected post LLIN as measured by dissections and PCR. Likewise, while 0.7% of *An. punctulatus* were infective one year prior to LLIN (n=2996), none were infective post LLIN (n=675). The inclusion of vector control in the GPELF stands to improve the long-term durability of these programs.

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IMPACT OF HUMAN MOVEMENT ON MDA COMPLIANCE AND EFFECTIVENESS IN PAPUA NEW GUINEA

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The current program to eliminate lymphatic filariasis (LF) is based on the expectation that 4 to 6 rounds of annual mass drug administration (MDA) with population coverage ≥70% will result in unsustainable transmission. However, transmission has not been halted in some regions after reportedly achieving these outcomes. Possible reasons for MDA program failure could include incomplete drug coverage, missed MDA doses due to travel, or reintroduction of infected individuals following MDA. This study analyzes four years of weekly demographic surveillance records from a field trial of annual MDA in four communities of Papua New Guinea. Weekly in- and out-migration was recorded by local reporters performing active surveillance. Of 848 individuals in four communities, 48% were absent each year for an average of one month (range:1 week-10 months). This occurred at greatest frequency during times of agricultural activity. Of the people spending time away from their village of residence, 12-41% were absent during days of drug administration. 48-62% of these individuals received ≤2 of the 5 MDAs versus 40% of the general population. Following 4 rounds of MDA, 12% of mobile individuals and 5% of permanent residents were microfilaria positive (p=0.05). Results from this study may be used to better design MDA distribution programs and improve methods to estimate program coverage.

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WASTING IN EARLY CHILDHOOD AS A RISK FACTOR FOR STUNTING

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Childhood undernutrition is a risk factor for childhood illness and death. We obtained individual-level, longitudinal anthropometry data for 1,590 children 0-2 years old from nine cohort studies and found an association between stunting at 18-24 months and wasting during the first 18 months of life. In order to further explore the longitudinal relationship between wasting and stunting, we considered stunting status at 18-24 months as a function of wasting in the age intervals of 0-5, 6-11, and 12-17 months using GEE for logistic regression and robust variances. We found that children with their first wasting measurement in the 0-5, 6-11, and 12-17 month age groups were 1.3, 2.7, and 3.1 times, respectively, more likely to be stunted at 18-24 months than children with no wasting in any time period. In addition, using a random effects model and robust variances with length-for-age z-score (LAZ) at 18-24 months as the outcome measure, children with their first wasting in the 0-5, 6-11, and 12-17 month age groups had LAZs that were 0.2, 0.6, and 0.9 z-scores lower, respectively, than those with no wasting during any of those periods. We also considered multiple periods of exposure and found that children with more recent wasting, as well as wasting in more than one 6-month age group, were more likely to be stunted at 18-24 months and to have lower LAZ scores than children who were never wasted during follow up or who were wasted only in the 0-5 month age group. Finally, since variability in weight-for-length z-scores (WLZ) due to seasonality of infection or food insecurity may result in decreased linear growth, we modeled the impact of WLZ variability during the first 18 months on LAZ at 18-24 months. Children with greater WLZ variability were more likely to be stunted and were shorter by 0.3 z-scores at the end of follow up. The results of this study indicate that children with highly variable WLZ are at particular risk for stunting and actions should be taken to decrease that variability. In addition, targeted interventions to decrease wasting in young children are likely to decrease stunting prevalence overall.

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HOW LONG DOES GROWTH IMPEDE IN CHILDREN AFTER ACUTE DIARRHEA?

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Diarrhoeal disease is the second leading cause of mortality and morbidity globally. The disease kills 1.5 million and causes 2 billion episodes of diarrhoea each year and is considered as the leading cause of malnutrition in <5 year age children. We evaluated whether moderate to severe diarrhoea has short and long term nutritional sequelae. In a case control study conducted at the rural Basse of The Gambia in Western Africa we measured the anthropometric indices of the <5 children presenting with signs of moderate to severe diarrhoea. Age and sex matched controls were enrolled from the community within a demographic surveillance system. Cases and controls were then followed up at 60-90 days and 18-24 months after the enrolment. Nutritional assessment was based on the WHO's Z scoring system. We enrolled 854 cases and 1161 controls during the two years of the study. According to the weight for age Z score (WAZ), the case children were significantly (p<0.001) more severely malnourished compared to controls [OR 3.59 (95% CI 2.65 to 4.88); OR 1.98 (95% CI 1.21 to 3.26); OR 3.54 (95% CI 2.18 to 5.78); OR 11.59 (95% CI 5.39 to 25.59); for all children, 0-11m, 12-23m and 24-59m age group respectively. A similar trend was observed on the weight for height/length Z score (WHZ). On height/length Z score (HAZ) only the 24-59 months age group of cases were significantly different to controls [OR 2.84 (95% CI 1.42 to 5.68) p 0.001]. On 60-90 day follow up a higher proportion of case children remained severely malnourished on WAZ, HAZ and WHZ scale and a significant difference was observed in WAZ and HAZ in the older age strata [WAZ-OR 2.68 (95% CI 1.17 to 6.16), p0.017; HAZ-OR 4.37 (95% CI 2.03 to 9.52), p<0.001]. Children aged <5 years fail to regain their growth compared to their peers after acute diarrhoea. A nutritional rehabilitation after an acute episode of diarrhoea is highly recommended. These cohorts need to be followed up till the preschool age to understand the long term growth faltering and their consequences in this population.

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PARASITISM IN CHILDREN AGED THREE YEARS AND UNDER: EFFECTS ON GROWTH AND VACCINE RESPONSE IN RURAL COASTAL KENYA

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Children are at high risk for helminth and protozoal infections, although estimates of parasitic burden and effects on growth are lacking in those under age 5 years. Recent evidence shows that parasitic infections may also alter vaccine response. Our objective was to document the prevalence of parasites and their effects on growth and response to childhood vaccines in young children in coastal Kenya. Stool, urine and blood samples were collected from children at 6 month intervals until age 3 years and tested for soil transmitted helminths (STH: Ascaris, Trichuris, hookworm, Strongyloides), protozoa (malaria, Giardia), and schistosomiasis. Height, weight, and head circumference (HC) were measured at each visit. Prenatal maternal helminth and protozoal infections were documented. Response to tetanus, diphtheria, hepatitis B virus, Haemophilus influenzae type B, and poliovirus vaccinations were measured by standard ELISA. McNemar's test, Student's t test on log transformed titers, and repeated measure modeling were used to analyze data. Of 545 children, 32% had parasitism and 8% had polyparasitism. Hookworm was most prevalent STH (11%), followed by Trichuris (10%), Ascaris (4%) and Strongyloides (2%). Giardia was the most prevalent protozoan (13%) followed by malaria (12%). 4% had schistosomiasis by IgG4 testing. Early childhood infection with STH, hookworm, and malaria were associated with maternal infection. Polyparasitized children were more likely to have polyparasitized mothers (p=0.01) and have poor HC growth rate (0.002). Children with hookworm (p=0.01), any STH (p=0.049) or any parasitic infection (p=0.039) had slower weight gain. Children with hookworm (p=0.04), Giardia (p=0.03), Strongyloides (p=0.001), schistosomiasis (p=0.02) or malaria (p=0.01) had slower HC growth rates. Children with hookworm, Trichuris, or Giardia had statistically lower tetanus titers than uninfected children; those with malaria or any parasitic infection had statistically lower diphtheria titers. Our results document the under recognized burden of parasitism in children aged 0-36 months in rural Kenya. Parasitic infections in this young age group have detrimental effects on weight, height, and HC growth rates and may have significant implications on child health. Certain parasitic infections in childhood, such as STH and malaria, may also be linked to decreased response to standard childhood vaccinations.

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A CLUSTER-RANDOMIZED EVALUATION OF A RESPONSIVE FEEDING AND STIMULATION INTERVENTION ON NUTRITION AND DEVELOPMENT OUTCOMES IN RURAL BANGLADESH

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Parenting education for feeding and stimulation of infants and young children is needed, particularly in South Asia, where 45% of children are malnourished and many do not achieve their learning potential. Responsive stimulation is sometimes associated with better language development. Six group sessions with mothers and young children in rural Bangladesh used demonstration and coached practice to promote responsive stimulation, feeding and hand washing. Compared with an informational control, children in the intervention group had better developmental and nutritional outcomes, including language, hand washing and mouthfuls eaten. This study was undertaken to determine whether a responsive feeding and stimulation intervention improved nutritional and developmental outcomes compared to a regular information-based parenting program. A cluster randomized trial was carried out with 302 children 8-20 mo and their mothers in rural Bangladesh, randomized by village to one of three groups. The control mothers received 12 informational sessions on health and nutrition. The intervention groups received the same 12 sessions plus 6 sessions delivered by peer educators that included modeling and coached practice in self-feeding, hand washing and verbal responsiveness with the child during play. A second intervention group in addition received 6 months of iron-fortified Sprinkles. Nutritional outcomes included weight, height, self-feeding and mouthfuls eaten. Developmental outcomes included HOME Inventory, mother-child responsive talk, and language development. Analysis of covariance compared the three groups at posttest and follow-up, covarying pretest levels and confounders. Responsive feeding-stimulation groups attained greater weight-for-age, mouthfuls eaten, hand-washing, HOME scores, responsive talking, and language. No additional benefit was derived from Sprinkles. A brief behavior change program providing on modeling and practice in feeding and stimulation was found to benefit children's nutrition and language development. Sprinkles may have been insufficient to have an effect on these malnourished children.

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ACCEPTABILITY - A NEGLECTED DIMENSION OF ACCESS TO HEALTH CARE: FINDINGS FROM A STUDY ON CHILDHOOD CONVULSIONS IN RURAL TANZANIA

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Acceptability is a poorly conceptualized dimension of access to health care. Using a study on childhood convulsions in rural Tanzania, we examined social acceptability from a user perspective. The study design is based on the premise that a match between health providers' and clients' understanding of disease is an important dimension of social acceptability. For example, childhood convulsions may not be linked with malaria and hence local treatment practices may be preferred by mothers. The present study was linked to health interventions with the objective of bridging the gap between local and biomedical understanding of convulsions. The study combined classical ethnography with the cultural epidemiology approach using the EMIC (Explanatory Model Interview Catalogue) tool. EMIC interviews were conducted in 2007/08 (n=88) and results were compared with those of an earlier study in 2004/06 (n=135). The match between local and biomedical understanding of convulsions was already high in the 2004/06 study. Specific improvements were noted in form of: (1) a 46% increase among those who reported use of mosquito nets to prevent convulsions, (2) a 2 to 13 % decrease among caregivers who associated convulsion with 'traditional causes', and (3) a 14% increase in prompt use of a health facility. Such changes can be largely attributed to interventions which explicitly aimed at increasing the match between local and biomedical understanding of malaria. The match between local and biomedical understanding of disease is fundamental for successful control and management of health problems. Health interventions should take existing local knowledge and treatment practices into account and involve communities at all stages of interventions. In return, it is clear that well ingrained traditional beliefs can be modified with communication campaigns, provided that this change resonates with the beneficiaries.

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DELIVERING INTEGRATED CASE MANAGEMENT OF CHILDREN IN UGANDA THROUGH A TWO TIER SOCIAL FRANCHISE LINKING CHWS WITH PRIVATE CLINICS

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In Uganda, most efforts to improve health of children under five are delivered via the public sector and yet recent studies show that 60-83% of the population seek initial care from private providers. Community interventions that result in early diagnosis and treatment from trained providers in the public or private sector could significantly reduce severe death and disease among children under five. PACE launched an integrated community case management program in Mubende district, Uganda in November 2010 based on village health volunteers (VHTs) referring mothers of sick children to public health facilities or private facilities forming part of an existing social franchise network. The intervention package included training of VHTs in assessing and referring sick children as well as counseling mothers in appropriate prevention and treatment practices and training and supplying private providers with subsidized treatment for malaria, pneumonia and diarrhea. VHTs serve as a link between the community and trained private providers stocked with affordable quality treatment. A management information system (MIS) was created to provide data for continuous program improvement. MIS data show increases in children under five presenting at network outlets from 296 in quarter one (Q1) to 749 in quarter two (Q2). Results on the change in the rate of treatment of children presenting with symptoms of malaria, diarrhea and pneumonia as a result of VHTs promoting increased access to affordable treatment provided through local private providers, in addition to existing public sector services will be presented and discussed.

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FACTORS ASSOCIATED WITH THE TREATMENT QUALITY FOR ILL CHILDREN SEEN BY HEALTH WORKERS TRAINED TO USE INTEGRATED MANAGEMENT OF CHILDHOOD ILLNESS (IMCI) GUIDELINES IN BENIN

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A key aim of the World Health Organization's IMCI strategy is to improve treatment of the leading causes of child deaths in developing countries. Although studies have found that training health workers (HWs) to use IMCI clinical guidelines improves treatment quality, these same studies also identified important deficiencies. To improve performance, a clearer understanding of factors influencing HW practices is critical. We analyzed >9000 outpatient consultations performed by a cohort of 32 IMCI-trained HWs in Benin. We examined associations of HW- and patient-level factors with recommended treatment (i.e., prescriptions perfectly matched IMCI guidelines) for children 2-59 months old with at least one potentially life-threatening illness (e.g., malaria/febrile illness, anemia, pneumonia, or diarrhea). Detailed assessment, diagnosis, and treatment data were abstracted from specially designed IMCI registers over a 14-month period after IMCI training in 2001. We analyzed clinical findings recorded by HWs and classified 8277 children as having at least one potentially life-threatening illness (77.2% with malaria, 34.4% with anemia, 30.4% with pneumonia, and 16.5% with diarrhea). On average, 63.7% of children received recommended treatment, although performance varied widely by individual HW (range: 14.7-87.5%). Logistic regression modeling revealed that treatment quality was significantly poorer for children: seen by older HWs (each year reduced odds of recommended treatment by 4.1%; p=0.01), >12 months old (odds ratio [OR]=0.55; p<0.0001), with more complex illnesses (OR=0.95 per additional IMCI task required [range: 18-43 tasks], p<0.0001), with a danger sign (OR=0.33, p=0.0001), and with anemia (OR=0.27, p<0.0001). Prior supervision was not significantly related to the outcome. Findings illustrate how factors outside managers' control (e.g., clinical complexity) can be important influences on performance. Quality improvement strategies, such as audit and feedback, job aids, or targeted training, which are within managers' control, should address these weak points.

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EPIDEMIOLOGICAL SURVEILLANCE OF *BURKHOLDERIA PSEUDOMALLEI, ORIENTIA TSUTSUGAMUSHI* AND *RICKETTSIA TYPHI*, USING SEROLOGY IN BANGLADESH

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Melioidosis (*Burkholderia pseudomallei* infection), Scrub typhus (*Orientia tsutsugamushi* infection*)* and Murine typhus (*Rickettsia typhi* infection) are endemic in countries in South and Southeast Asia, but this has yet to be demonstrated systematically in Bangladesh. A simple and rapid means of estimating the prevalence of exposure to these organisms is measurement of antibody levels in a representative sample of the population. A prospective, cross-sectional, hospital-based serological survey was conducted in June 2010 at 6 major hospitals in 4 of the 7 divisions of Bangladesh. Age, gender, occupation and residential address were recorded for each patient. The presence of antibodies to *B. pseudomallei* was detected using the indirect haemagglutination assay and antibodies to rickettsioses (*O. tsutsugamushi* and *R. typhi*) were detected using enzyme-

linked immunosorbent assay. Of 1,244 patients enrolled, 359 (28.9%) were positive for *B. pseudomallei*, 146 (11.7%) for *O. tsutsugamushi*, and 579 (46.5%) for *R. typhi*. Farmers had an increased risk of seropositivity to *B. pseudomallei* (RR=1.4, 95%CI 1.0-1.8,P=0.027). Seropositivity to *R. typhi* was found to be commoner in farmers (RR=4.3, 95% CI 3.4-5.4, P<0.001), service workers (RR =3.9, CI 3.1-4.9 P<0.001) and housewives (RR=1.2, 95% CI 1.2-1.4, P=0.005). Optical density of ELISA to *R. typhi* correlated strongly with age (P<0.001).There were no other associations between antibody titre and age or seropositivity and occupation, gender or residence in a rural versus urban area. There was no clear geographic clustering of seropositives. In conclusion, rates of seropositivity to *R. typhi and B. pseudomallei* in Bangladesh were considerably higher than previously appreciated. These three organisms should be considered as possible causes of undifferentiated febrile illness in Bangladesh. Further studies will be needed to establish the incidence of clinical disease and distribution of environmental risk.

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ASSESSING THE RISING CASES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*: HOSPITAL AND COMMUNITY-ASSOCIATED CASES

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has since become a major cause of illness and death in our healthcare setting. Risk factors for HA-MRSA include hospitalization, older age, invasive devices, and residence in long-term care facility, including exposure to antimicrobial agents. HA-MRSA isolates are often resistant to several antimicrobial drug classes in addition to beta-lactams. The CA-MRSA infections usually affects young, healthy persons and associated with sharing towels or athletic equipment, participating in contact sports, living in unsanitary and crowded areas, using illegal intravenous drugs. Directions were given out for clinical microbiology laboratories to submit invasive isolates of MRSA to our unit, where we perform antimicrobial drug susceptibility tests on all isolates and characterize all isolates that were resistant to <3 non-beta-lactam antimicrobial drug classes. Most isolates were obtained from blood cultures. The full model for predicting invasive infection with CA-MRSA compared with HA-MRSA included age, seasonality, and hospital exposure, plus specimen type. The only significant predictors of CA-MRSA infection compared with HA-MRSA were age <69 years, which was associated with increased risk ([OR] 5.1, 95% [CI] 2.06-12.64), and hospital exposure (OR 0.07, 95% CI 0.01-0.51), which was associated with decreased risk. Most patients were hospitalized for their infections and the proportion of patients admitted to intensive care units did not vary by strain. Patients infected by MRSA were younger than those infected by other strains. The number of invasive MRSA infections reported and the number of invasive infections caused by CA-MRSA is on the increase. The increase of CA-MRSA poses a unique public health threat. It is now clear that CA-MRSA no longer causes only SSTIs but now causes an increased proportion of invasive infections in a rural state.

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ARE DIFFICULT-TO-REACH CHILDREN MORE LIKELY TO HARBOR TRACHOMA INFECTION?

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Mass antibiotic distributions are a major strategy for trachoma elimination. As programs in developing countries near elimination, there is concern that children who are difficult to reach may be more likely to harbor ocular strains of chlamydia that cause blinding trachoma. Here we compare infection in children who attended the initial day of monitoring versus those who presented on subsequent days. One year after administration of the third annual mass azithromycin treatment for trachoma, we performed conjunctival swabbing on a random sample of children in 12 Ethiopian communities. We defined those children who participated on the initial day as "easy-to-reach" and those who were only found on subsequent days as "difficult-to-reach." Subsequent monitoring days were necessary if all children were not present on the first day, which allows for comparison between easy-to-reach and difficult-to-reach children. Most communities required more than 1 monitoring day (10 of 12 communities). 584 children in total were assessed for the presence of chlamydial infection. On average, 15.9% (95% CI 8.8 - 26.6) of children were examined on a subsequent day. Evidence of chlamydia was found in 7.1% (95% CI 0.4 to 13.7) of children. Difficult-to-reach children were significantly less likely to have ocular chlamydial infection compared to easy-to-reach; Mantel-Haenszel common OR = 0.00 (95% CI 0.00 - 0.77). In this trachoma-endemic setting, difficult-to-reach children were less likely to harbor chlamydial infection, perhaps because those with more disease presented preferentially. This suggests that extreme efforts to achieve higher antibiotic coverage may not effectively reduce trachoma burden.

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CHARACTERIZATION OF STRAIN DIFFERENTIATION OF GENES IN *ORIENTIA TSUTSUGAMUSHI* USING MULTI-LOCUS SEQUENCE DNA TYPING (MLST)

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Orientia tsutsugamushi is the etiologic agent of scrub typhus or, more correctly, mite-borne typhus, in the Asia-Pacific region and most recently in South America. It is an obligate intracellular parasite transmitted vertically between mite generations and incidentally to humans via the bite of chiggers primarily of the genus *Leptrombidium*. Using the cell surface 56 KD gene *O. tsutsugamushi* has been well characterized as highly diverse, at least partially explaining the difficulty of developing effective vaccines. However the relationship between variation in the 56 KD type specific antigen gene and overall genome differentiation is unclear. Few studies have been reported examining the heterogeneity of more conserved housekeeping genes. This study examined the utility of multilocus sequence typing (MLST) to elucidate the diversity of *O. tsutsugamushi*. MLST was performed using PCR products amplified from purified rickettsial DNA's selected from isolates originally collected in Japan, Thailand, Burma, New Guinea, and South Korea. The strains chosen include representatives from most of the nine significant genetic subgroups within *O. tsutsugamushi* that have been identified based upon genetic differences in the 56 KD type specific antigen gene. Using the two published reference genomes, primers were developed for ten genes, i.e. *gpsA, mdh, nrdB*, *nuoF, ppdK, adk, lipA, lipB, sod*, and *groEL*. Preliminary data indicate the average pair wise distance of these genes is 2.1 per cent. This suggests that these genes may discriminate between strains and be used to construct clonal complexes within this species. Establishment of MLST protocols for *O. tsutsugamushi* could be used to characterize clinical isolates especially in regions where scrub typhus appears to be emerging.

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RISK FACTORS FOR PLAGUE MORTALITY - UGANDA, 2008-2010

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Plague is a severe, life-threatening disease. Over 95% of cases worldwide are reported from rural Africa. Although treatable, mortality rates range from 10% in developed countries to 40% in underdeveloped countries. We evaluated surveillance data and conducted a case-control study to evaluate risk factors for plague mortality in Uganda. A suspect plague case was defined as rapid onset of fever and painful lymphadenopathy or hemoptysis in a person presenting to one of the collaborating10 clinics or 2 hospitals in the plague endemic region of Uganda during January 2008 - December 2010. The case-control study, conducted during November 2008 - December 2010, included suspect plague patients and any deceased person whose death was recognized within 48 hours and suspected to be due to plague. We administered a questionnaire to study participants or their designee to assess knowledge, beliefs, attitudes, and behaviors. We compared the frequency of risk factors among patients with laboratory-confirmed plague using Chi-squared analyses. Among 199 suspect plague patients, 59 (32%) had laboratory-confirmed illness; 16 (27%) died. There were no significant differences between those who lived or died with respect to age or sex. Among 51 bubonic plague patients, the mortality rate was higher in those with cervical (4/5, 80%) vs. inguinal (9/36, 25%) manifestations (p=0.01). Twenty-six laboratory-confirmed plague patients were enrolled in the case-control study; 7 (27%) died. Patients who did not suspect plague as a cause of their illness prior to presentation or death were more likely to die (6/7, 86%) than live (7/19, 37%) (p=0.04). The median time from symptom onset to clinic presentation was 3 days and 1 day in those who died versus survived, respectively. The median travel time to the clinic did not differ between groups. Plague can be treated successfully if diagnosed early. Knowledge and suspicion of plague may result in reduced mortality in Uganda. Health care access did not appear to be a factor. These findings suggest plague education may reduce mortality in Uganda.

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ADAPTIVE IMMUNE RESPONSE TO *BRUCELLA SPP.* IN HUMANS

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Brucellosis is an ancient zoonotic disease that still represents a significant public health problem in Georgia. Infection is usually linked to exposure to infected animals and/or consumption of unpasteurized animal products. In addition, *Brucella* species are considered a substantial threat as possible biologic weapons and are included on the CDC list of possible bioterrorism threat agents. Diagnosis of brucellosis is often reliant on detecting immunological evidence of exposure to specific antigens using antibody-based blood tests. A variety of commercial kits and protocols exist for the measurement of *Brucella* specific antibodies. Despite this, our understanding of the host immune response to this disease is relatively limited and consequently there is a need for further research. In this study we examined humoral immune responses in 43 individuals diagnosed with brucellosis 3 to 12 months before enrollment, many of whom still had persistent symptoms after completion of initial therapy. Sera from 35 of 43 patients had antibodies that bound to *Brucella* lipopolysaccharide (LPS) by COMPELISA and 34 of 38 patients had demonstrable specific antibody to brucellergene OCB antigens; results from the two ELISAs were highly correlated ($p = 0.031$, $R = 0.851$). We also studied cellular immune responses in 15 patients, all of whom generated interferon (IFN)-γ in response to *ex vivo* stimulation with *Brucella* protein antigens and the majority of whom maintained measurable humoral responses to both LPS and protein antigens. From this initial study we conclude that measurement of antibody and cellular IFN-γ responses to brucellergene OCB protein epitopes may be worthy of further investigation as an alternative or adjunct to current diagnostics.

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IMMUNE RESPONSES TO PLAGUE INFECTION IN WILD *RATTUS RATTUS*, IN MADAGASCAR: A ROLE IN FOCI PERSISTENCE?

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Plague is endemic within the central highlands of Madagascar where the black rat, *Rattus rattus*, is the main reservoir. Rat immunity could play an important role in the stabilization of the foci. However, immune responses *of R. rattus* against *Yersinia pestis* are poorly investigated. Here we experimentally infected wild rats with *Y. pestis* to investigate short and long-term antibody responses. High levels of anti-F1 IgM and IgG were found in rats one and three weeks respectively after challenge, with responses differing between villages. In the long-term response experiment, a small proportion of rats had anti-F1 responses lasting more than one year. These findings may have implications for plague epidemiology. In addition, the results indicate that serological investigations in the field can detect outbreaks up to 6 months later. Comparing the ELISA and an anti-F1 antibody dipstick indicated the dipstick could be useful in the field.

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LEPTOSPIROSIS IN ACUTE FEBRILE PATIENTS IN GHANA: DIAGNOSIS BY CULTURE, SEROLOGY AND POLYMERASE CHAIN REACTION

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Leptospirosis is a zoonotic disease found in most tropical and temperate areas of the world. Humans contract Leptospira through exposure to environments contaminated by the urine of chronically infected animal sources mainly rodents, dogs, pigs and cattle. The burden of leptospirosis in Ghana is unknown or underestimated because it can mimic many diseases, e.g. malaria, dengue fever and other viral haemorrhagic diseases. In an ongoing Integrated Hospital-Based Infectious Disease Surveillance being conducted by the Ghana detachment of NAMRU - 3, in the Greater

Accra and Northern Region of Ghana, blood samples have been collected from 231 acute febrile patients meeting enrollment criteria.This study has been approved by the NMIMR and NAMRU-3 institutional review boards. 2 drops of the blood are inoculated in EMJH medium for culture and Serum is separated from the plain blood samples. So far, 180 of the serum samples have been tested for IgM antibody by ELISA and 40 for Leptospira DNA by PCR. Detection of antibody was done by Pan Bio Leptospira IgM ELISA, following manufacturer's instructions. Detection of DNA by lig-based Conventional PCR: Extraction of DNA was performed using the QIAGEN blood mini kit. Amplification of DNA: Primers used were designed from the conserved region of ligA and B. Culture results are not available now, since it needs more time. Serodiagnosis will be performed by the microscopic agglutination test (MAT). Of the 180, 14 samples (7.7%) were positive for the presence of IgM antibodies by ELISA and one was equivocal (0.55%). Conventional PCR demonstrated DNA in none of the 40 samples tested so far; also, ELISA positive samples were negative by PCR, proving PCR to be more sensitive. This suggests that almost 8% of the patients have been infected before. Leptospirosis is underreported in Ghana, where malaria is endemic, and because it is an emerging infectious disease in this part of the world, all diagnostic tools such as culture, MAT, ELISA, Conventional and Real time PCR (RT-PCR), should be explored to know the burden of this disease. In addition, improve treatment of patients in Ghana.

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ANTIBIOTIC RESISTANCE OF INVASIVE NON-TYPHOIDAL *SALMONELLA* (NTS) ISOLATES IN CHILDREN FROM WESTERN KENYA

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Community-acquired non-typhoidal *Salmonella* (NTS) bacteremia is widespread in *Plasmodium falciparum* holoendemic transmission regions of Africa. In these regions, NTS bacteremia complicates malaria and other childhood illnesses, and increases childhood mortalities. Our recent studies have shown that NTS is the most common cause of malaria-related bacteremia and enhances malaria severity and mortality in children from western Kenya. Clinical treatment of children with NTS infections is worsened by the rampant and increasing antimicrobial resistance. As such, the patterns of antibiotic resistance by bloodstream NTS isolates were investigated in children (n=67) from western Kenya with *P. falciparum* malaria (n=30), without malaria (n=20) and following acute febrile visits to hospital (n=17; malaria[+], n=1 and malaria[-], n=16). *Salmonella enterica* serotypes Typhimurium and Enteritidis were the only serotypes isolated using standard microbiological procedures, while *in vitro* antibiotic resistance defined by intermediate or full resistance was determined using the disc diffusion method. Results reveal that *in vitro* resistance to ampicillin/salbactum [60/67 (89.6%)]; amoxicillin/clavulanic acid [50/66 (75.8%)]; trimethoprim/sulfamethoxazole [58/67 (86.6%)]; chloramphenicol [52/67 (77.6%)]; ciprofloxacin [22/43 (51.2%)]; cefotaxime/clavulanic acid [9/29 (31.0%)]; nalidixic acid [7/30 (23.3%)] and gentamicin [14/64 (21.9%)] was common. Multi-drug resistance, based on resistance to three or more antibiotic classes, was also high [56/67 (83.6%)]. Additional analyses demonstrated higher ciprofloxacin resistance in children without malaria [13/15 (86.7%)] relative to those with malaria [7/18 (38.9%); P=0.011] and acute visits [2/10 (20.0%); P=0.002]. These results demonstrate that antimicrobial resistance to common antibiotic agents is high in this area, justifying an urgent need for clinical and public health surveillance.

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CHARACTERIZATION OF EXTREMELY DRUG RESISTANT ISOLATES OF *MYCOBACTERIUM TUBERCULOSIS* DETECTED IN COLOMBIA DURING 2006 TO 2010

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Extremely drug resistant tuberculosis (XDR-TB) represents an important threat to TB control worldwide. Resistance to isoniazid and rifampin, the two main drugs used for TB treatment, together with resistance to a fluoroquinolone and one of three injectable second-line anti-TB drugs, makes the XDR cases difficult to treat and cure. According to the World Health Organization, Colombia reported at least one XDR case since 2008, and nine XDR-TB cases have been detected in the past five years in Valle del Cauca. The aim of this study was to perform a molecular characterization of this set of XDR isolates, including a sociodemographic description of cases. XDR profile was identified using Proportion Method on 7H10 agar medium. Sociodemographic data and clinical outcome were obtained through local health authorities. The isolates were genotyped using spoligotyping and MIRU-VNTR 24 loci methodologies and mutations associated with resistance to first and second-line anti-TB drugs were detected using Genotype® MTBDR*plus* and *sl* assays (Hain lifescience). The majority of patients were male (56%) and patient's age ranged from 16 to 44 years with a median age of 30 years. Five out of the nine patients had a mortal outcome (56%). Spoligotyping identified three families: Beijing 190 (56%), H1 62 (11%), U 881 (11%) and two different orphan types (22%). All isolates were further classified into 7 genotypes using MIRU-VNTR. Moreover, three of the mortal cases were classified as Beijing 190, clustered as the same MIRU-VNTR genotype and also had the same mutations for the evaluated genes, which could suggest clonality. The S315T1 mutation in the *katG* gene for resistance to isoniazid and the S531L mutation in the *rpoB* gene for rifampin resistance were the most frequent (89% and 67%, respectively). D94G, A1401G and M306V mutations were found in the *gyrA*, *rrs* and *embB* genes (67%, 100% and 56%) associated to fluoroquinolones, aminoglicosides and ethambutol resistance, respectively. The high frequency of *katG* S315T1 and *rpoB* S531L mutations are consistent with previously reports from other South American countries. Genetic screening of these mutations may provide rapid detection of XDR cases and improve their treatment. The mortal Beijing 190 cluster identified in this study calls for further detailed studies including virulence factors, which may lead to novel drug targets.

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SEROTYPING INVASIVE PNEUMOCOCCAL MENINGITIS IN THE REGION OF BOBO-DIOULASSO

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Meningitis remains a major public health problem. The western Burkina Faso is in the African meningitis belt, with recurrent epidemics of bacterial meningitis. These outbreaks are usually caused by Neisseria meningitidis, a strainable alone to cause epidemics of meningitis. However, in recent years there has been an upsurge in cases of meningitis due to Streptococcus pneumoniae that occur throughout the year with a fatality rate of pneumococcal meningitis (~ 50%) 5-10 times higher than for Meningococcal meningitis in [7], but unfortunately very few studies show serotype profile of this germ in Africa. Our study aims to profile the seeds of pneumococcal serotypes circulating in the area of western Burkina Faso. Its main objective to participate in the microbiological monitoring of pneumococcus by monitoring the emergence of new types of germs, and the resurgence of invasive strains in the region of the high-basins. We also study the circulation and the biodiversity of strains of S. pneumoniae infections of pneumococcal meningitis in the western region of Burkina Faso. Patients with suspected meningitis were recruited between 2009 and 2010. Samples of cerebrospinal fluid were collected and analyzed by

standard microbiological techniques. Bacterial isolates were analysed by PCR. An increase in the incidence of pneumococcal meningitis has been observed from 2009 to 2010. Of the 154 samples of S. pneumoniae analyzed, the serotype 1 represented for more than 50% of germs from CSF analyzed with a high virulent lineage and a high propensity to cause meningitis; our results suggest that this strain may have the potential to cause an epidemic. Conclusion: These preliminary results show that the growing of this strain could be a potential epidemic. As perspective we need to investigate the virulence of this serotype 1 and others serotypes on the ability of invasive pneumococcal disease.

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MIXED INFECTION OF *CHLAMYDIA TRACHOMATIS* GENOTYPE L2 AND *CHLAMYDIOPHILA ABORTUS* IN PIGS

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In Ukraine, chlamydiosis is diagnosed in 73% from studied pig samples. In the first-time impregnated pigs the abortion rate is 45%; abortions in master sows are rare, they get usually 2-3 still piglets, lethality of others is 60%. Three aborted foetuses from the pig farm in Cherkasy region, Ukraine, were studied using microscopy and IFT. 5 day chicken embryos (CE) were charged with specimens of aborted foetuses. Experimental infection was performed using white mice, guinea pigs, and gnotobiotic piglets. CE, guinea pigs and gnotobiotic piglets were studied in genus-specific RT-PCR and microarrays (Alere) for detection of chlamydial species and genotyping of *Chlamydia* trachomatis. The pig farm had previous history of chlamydiosis for about 3 years. The dissection of aborted foetuses revealed hyperemia of brain vessels and liver dystrophy, petehia and spread haemorrhages in epicardium and kidneys. Microscopy and IFTs were positive. Chlamydiae (strain A-2536) were isolated on CE. The 6th CE passage induced death in 4.2 d.p.i. with the infectious titer 10-4,16 ELD50/0.4ml. The 5th and 4th CE passages caused 100% lethality in white mice, and 30-40% lethality and 100% abortions (in 5-6 d.p.i) in guinea pigs respectively. The 5th passage of isolated chlamydiae on chicken embryos did not cause visible clinical signs in gnotobiotic piglets in 35 d.p.i. with exception of 1 piglet with acute disease which dead on 21 d.p.i. Multiple pathological changes typical for chlamydiosis were found in dissection. Chicken egg yolk, organs of guinea pigs and gnotobiotic piglets were positive in genus-specific RT-PCR. Using the same DNAs, the microarray assay revealed mixed infection of *C. trachomatis* and *Chlamydophila* spp. in chicken egg yolk; *C. trachomatis, C. abortus*, and *Chlamydia* and *Chlamydophila* in 1 guinea pig; *C. trachomatis* and *Chlamydia* but no *Chlamydophila* in all gnotobiots. Microarray based genotyping of DNA from gnotobiots revealed genotype L2 of C. trachomatis.

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DEVELOPMENT OF ELISA FOR THE DETECTION OF LEPTOSPIROSIS SPECIFIC ANTIBODIES USING THE OUTER MEMBRANE LIPOPROTEIN LIPL32 AND LIPL41

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Leptospirosis is caused by spirochaetes of the genus *Leptospira*. It is considered to be the most widespread zoonotic disease in the world. Symptoms of leptospirosis are easily confused with a variety of other febrile illnesses (e.g., dengue and malaria) that require different treatment regimens. Currently, the Microscopic Agglutination Test (MAT) is the standard method for the diagnosis of leptospirosis. It is not only technically complex but also time-consuming. With the publication of the whole genome sequences of several pathogenic species of *Leptospira*, hundreds of genes encoding surface-exposed lipoproteins and outer membrane proteins were identified as candidates for the development of rapid diagnostics of leptospirosis. Among them, LipL32 and LipL41 are considered excellent candidates as they are present only in pathogenic strains and have been shown as surface exposed. Here, we prepared recombinant LipL32 and LipL41 proteins and showed that both were recognized by leptospirosis patient sera in western blot. Fifteen MAT confirmed positive sera were used to evaluate these two antigens in ELISA. Preliminary results showed 9 were IgG and 9 were IgM positive against LipL32. Eleven samples were IgG positive and 10 samples were IgM positive against LipL41, respectively. Some samples had specific IgM antibody against LipL32 only and some had specific IgM against LipL41 only but not both. These data suggested that both LipL32 and LipL41 antigens should be needed to improve the assay sensitivity and to develop rapid sero-diagnostic assays.

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HOW RESISTANT IS *STAPHYLOCCOCUS AUREUS* IN PEDIATRICS AT PONCE SAN LUCAS HOSPITAL?

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Community Acquired Staphylococcus aureus (CA-MRSA) infections, are usually, acquired by community persons without hospitalizations or surgical interventions during the year preceding the infection. Infections with CA-MRSA were first reported in the United States pediatric population during the 90's, mainly as a cause of skin and soft tissue infections. Frequency of CA-MRSA infections in Pediatric patients admitted to Hospital Episcopal San Lucas is unknown. Data shows that these infections doubled from 33% to 65% during 2004 to 2006. The objectives of our study were to determine resistance patterns of CA-MRSA skin and soft tissue infections, and associated morbidity in pediatric population admitted to hospital. This is a descriptive, transversal retrospective, IRB approved study was done. Cultures from all pediatric patients admitted to hospital (59) from January to December of 2008 were reviewed. Exclusions were made for infections other than those of skin and soft tissue, nosocomial or immune-compromised children (28). Localization of infection, antibiotics used, and changes in therapy were obtained (29). Our findings includes; children from 6 months to 3 years were more frequently admitted (13 cases/44%). In our sample almost 60% of admitted children with skin and soft tissue infections had CA-MRSA as per sensitivity pattern. A large portion of community physicians, still use penicillin derivatives, or first generation cephalosporins before or after lesion drainage even though CA-MRSA is highly resistant to these antibiotics. We conclude in our study that CA-MRSA is a frequent cause of skin and soft tissue infections in the pediatric population admitted to our hospital. Children ≤ 3years are most frequently affected. The high rate use of penicillin derivatives or first generations cephalosporins for treatment of skin and soft tissue infections by CA-MRSA, needs to be addressed, so community physicians become aware of the recommendations issued for proper management of these infections.

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EXPERIMENTAL LEPTOSPIROSIS IN HAMSTERS INDUCE HYPOMAGNESEMIA AND HYPERKALEMIA IN ACUTE-PHASE DISEASE

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Patients with severe leptospirosis develop hypomagnesemia during acute phase disease as well as sodium and potassium wasting defects. Non-oliguric renal failure rapidly evolves to an oliguric hyperkalemic form that is associated with a poor outcome. The renal waste of Mg2+, Na+, and K+ may be related to the production of nitric oxide (NO), which is

a known inhibitor of the Na,2Cl,K co-transporter of the thick ascending limb. Hamsters were experimentally infected with the virulent Leptospira interrogans serovar Copenhageni strain Cop (~ 6 fold the lethal dose 50%). Groups of five hamsters were euthanized at different intervals (4, 8, 16 and 28 days post-infection) and evaluated for kidney lesions and serum levels of NO, K+, Mg2+ and Na+. Hamsters were separated into four groups according to the treatment regimen started on the tenth day: ampicillin (AMP), methylene blue (MB), ampicillin and methylene blue (BOTH) and no treatment (NONE). MB is a known inhibitor of nitric oxide synthase. All groups exhibited increasing serum levels of K+ from day 4 to day 16. In addition, a significant decrease in the serum levels of Mg2+ was observed in all groups on day 8. Serum levels of Na+ remained unaffected by the treatment regimens, except for a decrease in the MB group on day 16. In conclusion, hamsters developed hypomagnesemia during the acute phase of experimental leptospirosis, which was not prevented by antimicrobial treatment or inhibition of NO production. Conversely, hypokalemia was not observed, as all groups showed increasing levels of serum K+. Furthermore, treatment with MB had no effect on Mg2+ and K+ serum levels during acute phase leptospirosis in hamsters.

1098

BACTEREMIA AND ANTIBIOTIC RESISTANCE IN ACUTE FEBRILE PATIENTS IN ACCRA, GHANA - A PILOT STUDY

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Malaria is a leading cause of morbidity in Ghana, accounting for 40-60 percent of cases in outpatient clinics. Unfortunately, malaria diagnosis is often inferred from subjective presenting symptoms rather than objective laboratory results. It is important therefore to establish the burden of non-malarial pathogens in acute febrile illness. The pilot study being described herein was undertaken to describe the burden of disease presenting as febrile illness in two Accra hospitals. One hundred and sixty four people were enrolled in this surveillance study. Patients presenting with fever lasting two days or more and a temperature of >38°C were recruited. Individuals with an obvious focal clinical diagnosis like diarrhea, respiratory or urinary tract infection, cellulites and or rheumatic fever and children under the age of 4 years were excluded. Enrolled cases were those that had met the case definition and given informed written consent. This study was approved by the Noguchi Memorial Institute for Medical Research and Naval Medical Research Unit-3 institutional review boards. Data on sex, age and recent exposure to rodents, pets or domestic animals was recorded and 7-10 ml of venous blood drawn for serology, culture and malaria thick film tests. All isolates were identified, while the malaria film results were obtained from the hospital laboratories. A total seven (4.27%) bacteria were isolated and identified; two *Salmonella typhi,* one group A *Streptococcus,* one Salmonella Group B and three *Staphylococcus aureus*. One of the bactaeremia positive blood samples was malaria smear positive, while five were negative and one was not tested. The clinical diagnosis for five of these was malaria. Four isolates out of the eight were found to be resistant to Ampicillin while one *S. aureus* showed resistance to Ampicillin, Penicillin and Oxacillin. The *Salmonella* group B isolate was resistant to Chloramphenicol, Ampicillin and Sulfamethoxazole/ Trimethoprim. This information suggests that bacterial infections are responsible for at least one out of every twenty five presumed malaria cases. Additionally, viral etiologies ought to be given consideration when patients with febrile disease present at our hospitals. In order to get a clearer picture of agents of febrile disease in Accra, a larger study which includes additional hospitals, evaluation for viral pathogens and children under the age of four, is in the process.

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PREVALENCE AND RISK FACTORS OF LEPTOSPIROSIS AMONG RICE FARMERS OF ENDEMIC AREA IN A TROPICAL REGION OF PERU

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Leptospirosis is a zoonotic infection of major impact in tropical regions. We conducted a seroepidemiological cross-sectional study in the Valle del Alto Mayo, an endemic area of leptospirosis in the Peruvian Amazon, in order to identify the prevalence, local serovars, and associated risk factors of leptospirosis. 261 rice farmers were randomly selected to participate. Overall, leptospirosis infection was found to be 64.75% (95%CI: 58.76-70.74). The prevalence of specific serovars identified by microscopic agglutination (MAT) included: *Leptospira icterohaemorrhagiae* (34.5%), *L. autumnalis* (19.5%), *L. panama* (13.0%), *L. australis* (12.6%), *L. grippotyphosa* (8.4%), *L. bataviae* (5.4%), *L. djasiman* (4.9%), *L. pyrogenes* (3.8%), *L. cynopteri* (2.3%), *L. pomona* (1.9%), *L. georgia* (1.5%), *L. canicola* (1.1%), for *L. borincana*, L. *bratislava*, *L. ballum*, *L. wolfii* and *L. javanica* (0.76%), and *L. varillal* and *L. harjo* (0.38%). Among 169 positives cases, 84 (49.7%) were positive for one serovar, 58 (34.3%) for 2, and 27 (16.1%) to 3 or more. In addition, 35 of 169 (20.7%) positives cases were IgM positive, meaning they were in the acute phase of infection. Risk factors of infection included male sex (OR = 3.16, p=0.001), age between 30 to 49 years (OR = 1.86, p=0.001), a low level of education (OR = 1.67, p=0.03), working barefoot (OR = 1.84, p=0.001), and handling rats in the field (OR = 2.24, p=0.003). In conclusion, there is a high prevalence of leptospirosis among rice farmers of the Peruvian Amazon, of whom many were positive for more than one serovar. It is necessary to implement prevention and control of leptospirosis in this region.

1100

METALLOBETALACTAMASES PRODUCING *ENTEROBACTER* SPP. STRAINS FROM THE CENTRAL HOSPITAL OF CUMANÁ, SUCRE STATE, VENEZUELA

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Carbapenems represent the therapeutic of choice in hospital-acquired infections caused by multiresistant Enterobacteria. However, metallobetalactamases (MBL)-producing bacterial strains, which are resistant to carbapenems, are increasing their frequency worldwide and constitute a threat to the health of patients, especially in developing countries. From the period of August 2010 and March 2011, three Enterobacter spp. resistant to imipenem and meropenem were isolated from the central Hospital of Cumana (HUAPA), Sucre state, whose susceptibility was assessed by the Kirby-Bauer disk-diffusion assay. The double-disc synergy test with Imipenem/Meroperem and EDTA-Sodium thioglycolate showed the presence of MBL in all of the strains of *Enterobacter*. These strains were also resistant to most betalactams, ciprofloxacin and trimethoprim-sulfamethoxazole, although two of the strains were sensitive to aminoglycosides and all of them were sensitive to tigecycline. Amplification by PCR of a 382 pb fragment, using primers specific for VIM-type MBL gene on the DNA isolated from those three strains, showed the presence of this gene. The amplification using both VIM-1 and VIM-2 types showed the expected fragment of 801 bp only for the VIM-2 type in all the strains. As far as we know, this represents the first report of a MBL-producing *Enterobacter* strain and the first report of VIM genes in this genus in Venezuela and only a few cases of infection

due to MBL-producing *Enterobacter* have been reported in the literature worldwide. This represents a major matter of concern for hospital authorities since Enterobacter spp. are significant causes of nosocomial infections and are intrinsically resistant to aminopenicillins, cefazolin, and cefoxitin due to the production of constitutive chromosomal AmpC betalactamases.

1101

MULTIPLE ANTIBIOTIC RESISTANCE IN CLINICAL ISOLATES FROM GHANA

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The ever increasing resistance to antibiotics is a serious worldwide problem which has implications for morbidity, mortality and health-care both in hospitals and in the community especially in a developing country such as Ghana. This study, therefore, looks at an *in vitro* antibiotic sensitivity pattern of 7 standard bacteria and 14 wild-type bacteria isolated using Kirby-Bauer disc diffusion method and the guidelines set by the National Committee for Clinical Laboratory Standard. Briefly, two to six hour cultures of the microbes in peptone water that had achieved the 0.5 McFarland standard turbidity were flooded over Mueller-Hinton agar and antibiotic disc aseptically placed on the surface of the agar, allowed to dry, before being incubated at 37 ºC for 16-18 hours. The antibiotics tested included; Amikacin (30 µg/disc), Ampicillin (10 µg/disc), Penicillin (10 iu/disc), Cloxacillin (5 µg/disc), Erythromycin (15 µg/disc), Tetracycline (30 µg/disc), Gentamicin (10 µg/disc), Cotrimoxazole (25 µg/disc), Chloramphenicol (30 µg/disc), and some of the newer generation antibiotics including Cefixime (30 µg/disc), Cefuroxime (30 µg/disc), and Cefotaxime (30 µg/disc). The study revealed that, 29% of the isolates were resistant to all the 12 antibiotics used, 14% were resistant to 10 antibiotics, 21% were resistant 9, 25% were resistant to 8, 8% were resistant 6 while 4% were resistant to 5 antibiotics. Interestingly, all the microbes were resistant to tetracycline, cloxacillin, ampicillin and penicillin while 91.7% were resistant to erythromycin, all being first-line antibiotics in Ghana. Thus, a very serious multiple resistant antibiotic pattern of bacteria exists in Ghana.

1102

AN EXPLORATION OF THE KNOWLEDGE, ATTITUDES AND PERCEPTIONS OF THE LOCAL, ADULT, NON-MEDICALLY TRAINED GRENADIAN POPULATION ABOUT CERTAIN ZOONOTIC DISEASES

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Zoonotic diseases represent a leading cause of illness and death from infectious diseases in humans. The objective of this research study was to explore the knowledge, attitudes and perceptions of the local, adult, non-medically trained Grenadian population about certain zoonotic diseases. The study consisted of a quasi-experimental design consisting of 450 participants, selected using a convenience sampling in the Grand Anse and the Carenage areas of St. George's, Grenada. A questionnaire was employed to collect data on the knowledge, attitudes and perceptions towards five zoonotic diseases (ringworm, leptospirosis, creeping eruptions, rabies and salmonellosis). The overall level of distribution of knowledge of zoonotic diseases was 38.6%. Knowledge of Ringworm (81.0%) was predominant among participants while leptospirosis and creeping eruption demonstrated the greatest deficiency in participants' knowledge. Knowledge of zoonotic diseases was found to have an effect on the attitudes and perceptions of persons towards the diseases. Education (p=0.0000) and income (p=0.0000) were found to be determinants of zoonotic disease knowledge while age (p=0.56) and gender (p=0.97) had negligible influence on the measure of knowledge, attitudes and perceptions.

1103

THE GEOGRAPHY AND ECOLOGY OF ANEMIA IN THE DEMOCRATIC REPUBLIC OF CONGO

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Anemia is a severe public health problem in the Democratic Republic of Congo (DRC). A better understanding of the spatial distribution of anemia as well as its causes can help the government to focus its prevention strategies. Using hemoglobin levels reported in the 2007 Demographic and Health Survey (DHS) for the DRC, prevalence estimates were generated and ecological drivers of malaria were explored using spatial statistical analyses and multilevel modelling. Of the 4638 female respondents aged 15-59 years, 29% were anemic (hemoglobin <11 g/dL); of the 526 pregnant respondents, 53% were anaemic. Regional variation in these rates was mapped using the inverse-distance weighting spatial interpolation technique. Pregnant women were 33% more likely to be anemic (p<.0001). Certain ethnic affiliations were associated with increased risk for anemia in pregnant women (p<.05). Older women (p<.05) were at increased risk, while pregnant women owning a refrigerator were 32% less likely to be anemic than other pregnant women (p<.05). Living in certain agricultural zones was protective while others increased risk for anemia. Neither malaria PCR positivity nor HIV seropositivity increased the risk of anemia. This research demonstrates the feasibility of using population-based demographic data and geographic methods to study nutritional deficiencies in a tropical setting. This study provides the most accurate population-based estimates to date of where anaemia occurs in the DRC and what factors contribute to the estimated spatial patterns.

1104

NOMADS ACCESS TO THE CURRENT HEALTHCARE SYSTEM IS IMPAIRED BY THEIR PERCEPTION OF ITS COST, QUALITY, ACCESSIBILITY AND BY GENDER SEGREGATION IN TIMBUKTU, MALI

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Access to community-based healthcare services is one of the key factors in successful public health policy. In Mali, community-based interventions do not reach nomadic communities because of their lifestyle. In order to determine a better healthcare strategy for these nomadic populations, we conducted a cross-sectional survey in the administrative region of Timbuktu in Mali consisting of interviews of key members of the communities and distribution of questionnaires to community members, health care providers, traditional healers, community leaders and the mothers of children of 5 years or less. Informed consent was obtained from all participants. A mixed quantitative and qualitative data analysis approach was used. A total of 520 individuals from two nomadic communities, Gossi and Ber, were included in the questionnaire survey. Twenty (4%) underwent an additional interview. Based on the questionnaire survey, inhabitants of the two nomadic communities were livestock breeders (27%), housekeepers (26.4%), local traders (11%), farmers (6 %) and artisans (5.5%). The median age of the study subjects

was 38 years (18-86 years). The participants from Gossi and Ber lived a mean distance of 22.4 km and 8 km from the closest health center, respectively. The major complaints with respect to healthcare access were cost (35.7%), distance to the health center (46.2%), the quality of the services provided (39.2.%) and the lack of finances or means of displacement (79.4%). About 67% of the participants visit traditional healers first when they are sick. More than 25% of the participants from the community stated that they will never accept to be examined by a health care provider of the opposite gender. In summary, it appears from the interviews that the nomadic population has health needs not covered by the current health delivery system. Tackling the method and organization of health care delivery by adapting them to the local lifestyle, culture and values could lead to significant improvements in this regard.

1105

CURRICULUM DEVELOPMENT AND OVERSEAS OPPORTUNITIES IN TROPICAL INFECTIOUS DISEASES FOR THE 21ST CENTURY

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A needs-based analysis of our curriculum for graduate and medical students demonstrated that the Department's curriculum was centered on approaches to infectious disease more appropriate for developed countries. However, our graduate students are keenly interested and motivated to learn more about infectious diseases in tropical areas. JABSOM's Problem Based Learning Curriculum for medical students emphasizes clinical reasoning with limited exposure to global health issues. Opportunities for international research experiences for medical and graduate students were limited. With this in mind, we set out to reorganize our infectious disease curriculum. We are in the final phases of developing a one-year certificate course that covers didactic, laboratory and clinical aspects of infectious diseases. Physicians completing the Department's certificate course and who have completed a 2-month clinical overseas rotation will be eligible to qualify for the CTropMed Diploma examination offered by the American Society of Tropical Medicine and Hygiene. The core courses for the master's and doctoral degrees have been reorganized and can be completed during the first year after admission to the program. We have partnered with the other departments to provide learning opportunities in epidemiology, biostatistics, nutrition, maternal-child health, water supply/waste water management, and sanitation. Our pharmacology faculty is participating in all aspects of infectious diseases including treatment, control, and the development of new drugs. The immunology faculty is emphasizing the interplay of the host with the infectious agent in the development of immunity and/or disease along with the theory and practicalities of vaccine development. Finally, overseas opportunities have been set up to provide field experiences for graduate students, medical students and practicing physicians in the Asia-Pacific. We hope the exchange of students and faculty will foster a greater cultural understanding of infectious diseases in the context of real world experiences.

1106

ANTIBIOTIC THERAPY AND HYGIENE MEASURES TO INTERRUPT CHOLERA TRANSMISSION IN A PRISON IN ST. MARC, HAITI

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Partners In Health (PIH) supports the Ministry of Health (MOH) in Haiti to provide comprehensive health care to the rural and urban poor. In October 2010 cases of acute fatal watery diarrhea amongst adults in St Marc, Haiti signaled the beginning of a cholera epidemic that is currently ongoing. Living conditions in the St Marc prison are severely overcrowded and sanitation conditions are very poor. PIH is engaged in long-term medical mobile clinic activities in the prison in support of MOH. This abstract describes the initial cholera epidemic in the prison. On November 14th 2010 St Marc prison reported the first inmate with cholera, he died the next day. On that day, the prison housed 315 inmates and during the peak of the epidemic held 411 prisoners in 14 cells, each approximately 4 meter [2]. A mobile team from PIH comprised of three physicians, one public health coordinator and two community health educators collaborated with prison and MOH authorities to intervene in both treatment and prevention roles. Treatment: 16 cases of cholera were diagnosed on the teams arrival; a cholera treatment unit was established inside the prison with two cells transformed into cholera wards. An inmate at the prison was a trained nurse's aide and she was engaged to provide overnight care of the patients. Prevention: Bottled water was provided to inmates and later water purification tablets were used for the prison well water; cleaners were sent daily to clean and spray the prison with 0.2% chlorine spray; doxycycline 300mg was prescribed as a single dose by mouth to all inmates and prison guards once per week for a period of one month; training for prison guards and health promotion activities were carried out in the prison. In the four weeks following the intervention, there were no subsequent deaths in the prison of cholera. A total of 26 cases were registered. Treatment activities continued for one month until no new cases occurred. Early in the course of a cholera epidemic, close attention should be paid to hygiene and sanitation in prison settings to avoid unnecessary deaths. Antibiotic therapy and intense hygiene measures interrupted the initial cholera outbreak in St Marc prison.

1107

NOT AS SIMPLE AS IT SOUNDS: EVALUATION OF A BEHAVIOR CHANGE COMMUNICATION (BCC) INTERVENTION TO INCREASE PROMPT AND EFFECTIVE MALARIA TREATMENT IN CHILDREN UNDER FIVE IN KENYA

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In 2009, Population Services International in collaboration with the Ministry of Public Health and Sanitation launched a malaria behavior change communication intervention in Bondo district, Nyanza Province. The initiative aimed to improve: symptom recognition and prompt access to government clinics for febrile children; effective treatment with the recommended first-line drug artemether-lumefantrine (AL) in public health facilities; and adherence to the AL regimen by the child's caregivers. The intervention used various communication channels: road shows, radio spots, print media and community outreach to deliver 10 key messages. It was implemented between October 2009 and September 2010 and was evaluated through pre-post-intervention cross sectional household surveys. The surveys were undertaken in June/July2009 and July/August 2010. Households were selected using multi-stage cluster sampling and included in the survey if there was a child under 5. The primary outcome was the proportion of children under 5 with fever in the last 14 days accessing AL within 48 hours of fever onset. Logistic regression was used to test the association between the intervention and primary outcome. In the pre-intervention survey 600 households were surveyed giving 628 mothers with 958 children under 5 of whom 506 had been febrile in the past 14 days. In 2010, 700 households were surveyed containing 717 mothers with 1023 children under 5 of whom 515 had been febrile in the previous 14 days. The proportion of caregivers who sought any treatment for their febrile child within 48 hours increased between surveys [62.8% (59.1-66.4) vs 79.4% (74.8-83.3)]. However, there were no significant increases in the proportion of children accessing AL within 48 hours of fever onset [18.4% (15.0-22.3) vs 23.5% (19.5-28.0)] and there was a significant decrease in the use of government clinics. Logistic regression

on the 2010 data showed no association between exposure to the intervention messages and the primary outcome, however, knowledge of AL as the recommended treatment for uncomplicated malaria in children was significantly associated with prompt access to AL (OR: 4.3; 95%CI: 1.4, 12.7). The implications of these findings for the evaluation of BCC interventions, the relationship between knowledge and behavior and the complexity involved in attaining the roll back malaria target of 80% of malaria cases receiving prompt treatment with AL are discussed.

1108

COLLABORATION ON THE CONSTRUCTION OF A CLINICAL SITE FOR GLOBAL HEALTH EXPERIENCES

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Global Health and international medicine programs often take place in areas of the world that have limited access to health care services and scarce local resources which may require building rather expensive infrastructure to support the local program. Establishing partnerships is essential for the success of the initiative and to ensure sustainability. As part of the Global Health Honduras program of the Department of Family Medicine in the School of Medicine, and the developing Physician Assistant Program in the School of Health and Rehabilitation Sciences, both at Indiana University in Indianapolis, a clinical site is being built in collaboration with local Honduran, as well as American partners, both public and private. Construction is taking place on a donated property and work is done utilizing local resources by local laborers, which has brought some income to many of the families of the surrounding villages. International partners include the academic medical center, several charitable 501(c)3 organizations, and many individuals who have contributed time and resources towards its success. The soon to be completed medical center will serve multiple purposes: Besides being a clinical site for much needed local patient care and an international medical and health care education training center, it will also serve as a "communities center" to enhance the existing collaboration with local public health; and also as a hub for a communication and epidemiological surveillance center for surrounding isolated mountain villages which are medically under-served. Clinical care, health education and research to improve the quality of life for these residents is ongoing. This model of collaborative construction and future operation to serve the many needs of both local care and international medical and health care education is innovative as to address multiple needs, including enhancing interest in medical and other health care student's involvement in global health and international service learning, as well as involving multiple cooperating supporting agencies.

1109

MYSTERY CLIENTS AND DRUG COCKTAILS: FINDING OUT WHAT PATIENTS ARE REALLY BEING SOLD

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In Cambodia the first source of treatment for fever and other illnesses is often the nearest private provider - a private pharmacy or clinic, or most often, a informal drug seller. Previous studies have documented that the drugs are most often dispensed as little plastic bags containing a colourful "cocktail" of several different tablets and capsules. These appear to often contain antibiotics and antimalarials as well as antipyretics and vitamins, however their actual content has remained unknown. In order to identify accurately the contents of drug cocktails and to measure the frequency with which these are sold, we carried out a "mystery client" study. Actors presented to private providers with malaria-like symptoms, purchased drugs that were offered and documented the details of interaction, including whether or not they were offered a blood test or antimalarials. Over 200 interactions took place in 12 districts across Cambodia and the contents of the purchased cocktails are being analysed by mass spectrometry. We present the results of the analysis and discuss the implications for antimalarial and antibiotic resistance and patient safety as well as recommendations for policy and practice.

1110

POLIO ERADICATION IN PAKISTAN - THE LAST FRONTIER

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Continued poliovirus transmission in Pakistan poses a significant challenge to the Global Polio Eradication Initiative (GPEI). Pakistan has reported more polio cases than all other endemic countries combined for two years in a row. This burden is concentrated in a single geographical zone in the country's north-west, the Federally Administered Tribal Areas (FATA), reporting 74 (51%) cases in 2010. Military conflict in FATA has hampered immunization activities since 2008. Genetic homogeneity between viral isolates from FATA compared to 2 non-adjacent zones of persistent transmission, and other districts with recent outbreaks reaffirm this region's major polio reservoir status and highlight the presence of susceptible populations elsewhere in the country. We are conducting a comprehensive review of the Polio Eradication Initiative (PEI) in Pakistan, including a quantitative model, to explore reasons for failure at the district level. Pakistan reported 144 cases of polio in 2010 and 40 cases by May 23, 2011. An analysis of polio cases reported during 2009-2011 showed that 71% of cases had received no routine OPV doses and 32% had received no supplementary OPV (compared to 49% and 20% respectively in 2000-2002). Field observations of polio vaccination campaigns in Karachi showed many poorly motivated and under-paid vaccinators (paid only $1.7 per day), adolescents and children employed as vaccinators, variable quality of independent monitoring and lack of prior campaign publicity. Failure to vaccinate is the dominant explanation for continued transmission of polio in Pakistan. Lack of progress in polio eradication in Pakistan will lead to failure of the GPEI Strategic Plan 2010-2012. Heroic efforts to establish negotiated peace in FATA, massive increase in resources at the ground level, and involvement of local non-governmental partners to develop specific solutions for poorly performing areas are needed urgently if the goals of global polio eradication are to be met early in this decade.

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HOUSEHOLD EXPENDITURES DUE TO MALARIA CASE MANAGEMENT - UGANDA, 2009

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Malaria is the most frequently reported disease at both public and private health facilities in Uganda. Nearly 10 million cases of probable or confirmed malaria, the majority of which are in children under 5, were reported in 2009. Although access to effective treatment, including ACTs, has improved, such therapies are expensive and impoverished individuals continue to be disproportionately affected by illness. The RBM Partnership indicates that malaria "imposes a harsh economic burden on families who are least able to pay". The 2009 Uganda Malaria Indicator Survey (MIS) is a nationally representative household survey which provided coverage estimates of prevention and control activities. To assess the economic impact of malaria, we analyzed data on household malaria expenditures. We calculated the costs incurred for caring for a febrile child during the previous two weeks and performed both univariate and multivariate analyses. The MIS utilized a two-stage sample design; 4,421 households were randomly selected (response rate 97.5%) from 170 clusters. Of the

1667children under 5 with a reported fever in the prior 2 weeks, 1366 (81.9%) received medical care. Of these, 210 (15.4%) paid money for transportation, 394 (28.9%) for consultation, 758 (55.5%) for medicine, and 41 (3.0%) for hospitalization, with a mean total expenditure of $5.42 USD. The mean expenditure for ACTs ($2.54 USD) was lower than for other anti-malarial therapy ($3.21). A total of 230 (16.8%) had to borrow funds, 302 (22.1%) had to sell items, and 596 (43.6%) of caregivers took time off from their normal duties to care for their ill child (=4.87 days). Costs incurred at private and public health facilities were similar. Households in urban areas (p <.005) and belonging to higher wealth quintiles (p <.005) spent more money than their counterparts. Despite efforts to increase access to provide free and effective therapy in Uganda, the majority of caregivers incurred costs to care for their ill child. These costs were higher when compared with previous studies done during the era of monotherapy.

1112

AN EXAMINATION OF THE PHYSICAL AND SOCIAL CONSEQUENCES WOMEN WITH OBSTETRIC FISTULA EXPERIENCE IN THE DEMOCRATIC REPUBLIC OF CONGO

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Vaginal fistula is an abnormal connection between the woman's bladder and vagina (vesico-vaginal fistula) or between the vagina and rectum (recto-vaginal fistula), allowing urine or feces to leak uncontrollably. The condition most frequently occurs in women living in resource-poor countries who experience prolonged and obstructed labor. When not repaired, the condition can result in an inability to bear children. While attention has been given to traumatic fistula associated with gender-based violence in the east of the Democratic Republic of Congo (DRC), little is known about obstetric fistula and what happens to women affected. Qualitative research was conducted between March and June 2010 in 3 regions of DRC to 1) understand the characteristics of women with fistula; 2) examine physical and social consequences; and 3) compare programmatic approaches to aid women. Research methods involved key informant interviews (n = 15 participants), in-depth interviews (n = 33) and group discussions (n = 13). Women lived in remote areas, were on average under 20 years of age, had limited or no formal education, and had the condition for over 8 years. Results illustrate the extreme physical hardship and social vulnerability women face in a society where marriage and having children is critical to status and security. Women experienced physical sequelae from incontinence of urine and feces including offensive odor, sores and rashes caused by chafing, and urinary tract infections. Social consequences included marital dissolution, community rejection and ridicule, and limited economic productivity, impacting on women's mental well-being and forcing them to live an existence of isolation and shame. Results should guide policy makers in establishing treatment involving mobile surgical teams and increasing the surgical capacity of hospitals in all regions of DRC, as well as improving outreach to ensure that affected women obtain rapid treatment. Strengthening and increasing emergency obstetric services in rural areas are the best long term solutions to this devastating problem.

1113

KNOWLEDGE, ATTITUDES AND PRACTICES REGARDING MALARIA PREVENTION AND TREATMENT OF GOLD MINERS IN SURINAME

Hedley Cairo

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Currently malaria transmission in Suriname is primarily related to small-scale gold mining. The knowledge, attitudes and practices (KAP) of gold miners, regarding malaria has not been studied before. To be able to design and implement effective awareness interventions, it is necessary to first assess the environment in which the implementation will take place by studying current level of KAP. In July 2009 a cross sectional study was done in three selected locations. A questionnaire was administered to miners at the Tourtonne malaria clinic (n=112), in the Tourtonne neighborhood (n=27) and in a gold mining area (n=27). 77.1% of the respondents knew that malaria is transmitted by a mosquito. Only 37.9% cited mosquito net as a means of prevention. Only 28.3% claims to sleep under a net every night. 85.5% is concerned with malaria while in the interior. 84.9% knows that malaria can be fatal. Overall 44.5% of the respondents took a malaria test the last time they thought they had malaria. Of those working in Suriname 52.0% took test vs. 46.2% working in French Guiana. Overall 55.4% engaged in self-treatment, mainly because of no or difficult access to health facilities (92.0%). Overall, 64.9% completed their last malaria treatment; however, adherence was significantly higher when prescribed by health personnel (86.0%) compared to self-treatment (48.1%), p<0.001. 38.6% of the respondents do not receive any health information. To receive malaria information 25% prefers Brazilian TV, 26.9% information sessions in gold mining areas and 15.4% from health post. Only 11.5% prefers written media. Respondents were fairly knowledgeable about malaria transmission, but demonstrated poor adherence to treatment and behavior that is not consistent with effective protection from malaria. It is important to launch interventions that focus on creating awareness on the importance of drug adherence - to reduce the emergence and spread of drug-resistant malaria - and the use of effective protective measures. The best way to reach this group is through oral and visual media.

1114

MENINGITIS IN GHANA: A SOCIO-ECONOMIC STUDY OF THE IMPACT IN THE KASSENA-NANKANA DISTRICT OF NORTHERN GHANA

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Epidemics of meningitis occur throughout the world, but the greatest burden of disease is in the "meningitis belt" of the Sahel of Africa, where widespread epidemics occur every eight to twelve years. Knowledge, Attitudes and Practices (KAP) regarding meningitis and household Cost of the Illness (COI) are poorly understood and likely to show considerable variation across the belt. A KAP and COI survey was conducted in the Kassena-Nankana (K-N) district of northern Ghana, using a case-control methodology. Quantitative interviews were conducted with 74 cases and 148 controls. The COI was computed from patients' answers to questions about direct medical costs, direct non-medical costs and productivity lost due to meningitis. Results showed that there was high knowledge about stiffness of waist or neck (68%) as a symptom of meningitis by both cases and controls, but cases were more likely than controls to mention other critical early symptoms (high body temperature OR=0.44, vomiting OR=0.35, severe headache OR=0.52, loss of appetite OR=0.20).

There were no significant differences between the cases and controls with regards to the perceived causes of meningitis: heat was the most common cause mentioned by both cases and controls (82%). The average household cost of treating meningitis was $156 per case, which is higher than the average income of farmers ($87) in the district. Much of the total cost of meningitis was from productivity lost (60%); the average number of days lost due to meningitis was 29 days. The average cost of meningitis sequelae (i.e. hearing, neurological and vision problems) was $843 per case. In conclusion, knowledge of meningitis symptoms seems to be limited to stiff neck or waist, and a greater awareness of the causes and symptoms could be achieved with more focused education. The COI survey results suggest that meningitis poses a significant burden on households through out-of-pocket payment and lost productivity. Education and vaccination against meningitis will contribute to saving lives and palliate the economic consequences of the disease.

1115

COLLABORATION WITH TRADITIONAL HEALERS TO EXPAND SURVEILLANCE FOR PLAGUE IN NORTHERN UGANDA

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Plague, caused by the bacterium *Yersinia pestis*, is an important cause of morbidity and mortality in the West Nile region of northwest Uganda. Plague needs to be treated with appropriate antibiotics to reduce mortality. Estimates for Uganda suggest that 40-60% of the population use traditional medicine (WHO 2002). In this overwhelmingly rural, upland area with limited resources, traditional healers are an important component of local health care. This may contribute to inadequate treatment and underreporting of plague cases, and occupational risk for healers. Working with staff from the Uganda Virus Research Institute and the Uganda Ministry of Health, CDC undertook a qualitative assessment with a sample of traditional healers in 2009. The goals were to learn whether healers see suspected plague patients, to understand healers' knowledge of the disease, and to assess opportunities to involve traditional healers in the referral of suspected plague patients to clinics for life-saving antibiotic treatment. Eleven healers from two districts in the West Nile region were interviewed. Healers interviewed had general awareness of plague in their area and most indicated that they see patients with symptoms that could fit the description of plague. While most reported referring suspected plague patients to the local clinic, many also described administering "first aid" for a period of hours to days before referral. There was strong willingness to participate in training about plague and to engage in referral. General characteristics of the traditional healers' practice are also described, including use of herbs and witchcraft. Based on this assessment, a pilot referral network was put into place in 2010 with 10 traditional healers. Participating healers were trained through individual visits which included local clinic staff. They were provided a bicycle, referral cards and a cell phone programmed with minutes and clinic contacts. Initial results of this referral program will be presented.

1116

ACCEPTABILITY AND FEASIBILITY OF ELECTRONIC INFECTIOUS DISEASE SURVEILLANCE USING MOBILE PHONE TECHNOLOGY

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In India, physicians and other health-care personnel are increasingly using mobile phones and computers for daily communication and information management. However, application of these technologies to public health surveillance in India has been limited. We hypothesized that mobile-phone based electronic surveillance system will provide an acceptable alternative to standard, paper-based data collection forms that are typically used for collection of surveillance data in India. Rotavirus surveillance was established using a paper- and phone-based surveillance system for rotavirus diarrhea in and around Kolenchery, Kerala, India. We conducted a pilot study to evaluate the feasibility of implementing a mobile phone-based data entry system for rotavirus surveillance data collection. We surveyed current- and potential-users of the phone-based system using a structured questionnaire. A total of 186 hospital staff completed the survey including nine current and 177 potential users including nurses, administrative workers and physicians from eight hospitals. The mean age of current- and potential-users was 38.2 and 29.0 years, respectively. A total of 126 (68%) were physicians or nurses. Eighty-eight percent of respondents were willing to use the phone-based data collection system on a daily basis. The two most commonly cited concerns for regular use of the PDA in surveillance data collection were accuracy (34% potential *vs* 56% current users) and confidentiality of collected information. The mobile phones were capable of accurately displaying the data collection form and allowed for touch screen data entry and data storage using removable memory devices. In conclusion, our results suggest that mobile phone-based electronic surveillance data collection systems are acceptable and feasible for rotavirus surveillance in India. Scale-up and further evaluation of the phone-based system for surveillance data collection will now be developed in conjunction with local physician groups in India.

1117

THE IMPORTANCE OF AN INTEGRATED RESPONSE TO CHOLERA PREVENTION AND TREATMENT: PSYCHOSOCIAL SUPPORT TO SURVIVORS OF CHOLERA

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Along with the devastation the recent cholera outbreak in Haiti has brought, adding insult to injury to an already struggling country, has come stigma and anxiety around the origins of the disease and how it is transmitted. The sudden loss of life associated with cholera, and the inability to perform proper burials for those who have died, has brought back vivid memories of the tragic earthquake of January 2010, thus negatively impacting one's mental health. With a majority of Haitians living in areas without improved water sources and far from health centers, more than 5000 people have died and thousands more have been infected with the deadly bacteria. In mid-October 2010, Partners In Health/Zanmi Lasante (PIH/ZL), found ourselves in the midst of the epicenter of the outbreak, being the major partner working with the Haitian Ministry of Health's public hospitals along the Artibonite River valley. New to treating cholera, PIH/ZL quickly sprang into action, leaning on our multidisciplinary community-based model used for treating other diseases to guide in developing our rapid prevention and treatment response. As more people die and stigma against people with cholera

continues to rise, the psychosocial support team has developed a series of memorial services and support groups for families and individuals coping with the impact cholera has had on their lives. ZL psychologists have been leading memorial services to help with the healing process, but also to aid in reducing stigma. In the same vain, ZL developed support groups for survivors of cholera to help them regain their positive body image and aid in reintegration into their families and communities. Support group participants have noted a positive effect on their lives, giving them a shared space to discuss their experiences and support each other through their healing process. As the epidemic continues, the support groups and memorial services remain integral to the community response, working with traditional healers and community leaders to dispel myths surrounding the origins and spread of cholera.

1118

IN VITRO INVESTIGATION OF *PHYLLANTHUS FRATERNUS* AS AN ANTI-INFECTIOUS MEDICINAL PLANT

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In the last few years, a number of studies have been conducted in different countries to prove the antimicrobial properties of medicinal plants with high efficiency. It is in the spirit of continuing herbal medicine research that, the antimicrobial activity of aqueous extract from *Phyllanthus fraternus* was evaluated against seven standard and fourteen clinically important isolates using the agar-well diffusion method. In addition, the possible *in vivo* toxic effects from the extracts as well as the presence of phytochemicals were studied. Extracts from *P. fraternus* inhibited the growth of 5 out of 7 (71.4%) standard strains with zones of inhibition ranging from 0.0 to 29.67 ± 0.33mm while in the case of the wild strains, growth of 7 out of 14 (50%) strains were inhibited with zones of inhibition ranging from 0.0 to 14.33 ± 0.33mm. It was also observed that all the Gram positive bacteria (100%) were inhibited by *P. fraternus* whilst only 5 out of 15 (33%) Gram negative bacteria were inhibited. Thus, the growth of a total of 12 out of 21 (57%) microbes used were inhibited by the extract from *P. fraternus* with an average zone of inhibition of 9.37 ± 2.17mm. Significant phytochemicals detected were phenolics, polyuronides, reducing sugars, triterpenes and alkaloids. The LD_{50} value was found to be greater than 5000 mg/kg making P *P. fraternus* practically non-toxic according to Hodge and Sterner Scale.

1119

EVALUATING THE COST-EFFECTIVENESS OF CHECKLISTS AND TREATMENT ALGORITHMS: AN EMPIRIC EXAMPLE OF A MENINGITIS CHECKLIST IN RESOURCE LIMITED SETTINGS

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Checklists can standardize patient care, reduce errors, and improve health outcomes. In meningitis, with high patient loads, limited financial resources, and high mortality, CNS diagnostic algorithms may be a useful tool to guide diagnosis and treatment in resource limited settings. However, the cost-effectiveness of such algorithms is unknown. We developed a decision analysis model to evaluate 3 diagnostic strategies to assess the costs, diagnostic yield, and cost-effectiveness for CNS infections. Strategies were: 1) comprehensive "shotgun" approach of all available testing; 2) stepwise strategy with testing in a specific order; 3) minimalist strategy of high-yield testing only. Each strategy resulted in 1 of 4 meningitis diagnoses: bacterial, cryptococcal, TB, or other (aseptic) meningitis. In model development, we utilized published prevalence data from Cape Town, South Africa and published diagnostic test performance. We validated the 3 strategies in a prospective Ugandan cohort. The current comprehensive strategy resulted in 97% of patients with correct diagnoses at an average cost of $38.08/patient. The stepwise strategy had 93% correct diagnoses costing $15.74/patient, and minimalist strategy had 91% correct diagnoses costing $9.96/patient. The incremental cost effectiveness ratio was $308.03 per additional correct diagnosis for the stepwise over the minimalist strategy and $519.88 for the comprehensive over the stepwise strategy. As the proportion of negative lumbar punctures reached 50% (i.e. no meningitis), the costs increased to $21.32 per patient in the minimalist strategy; $30.49 in the stepwise strategy and $78.60 in the comprehensive strategy. Designing checklists and algorithms with consideration of both "effectiveness" and "costs" is essential. Through strategically choosing the order and type of testing coupled with disease prevalence rates and local medical practice, algorithms can be cost-effective and potentially sustainable in resource limited settings.

1120

PARTNERSHIPS IN FACILITY AND COMMUNITY-BASED RESPONSE TO A CHOLERA EPIDEMIC IN HAITI

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Partners In Health (PIH) supports the Haitian Ministry of Health (MOH) to provide comprehensive health care to the poor. In October 2010 cases of acute fatal watery diarrhea amongst adults in St Marc, Haiti signaled the beginning of a cholera epidemic. In its role supporting 12 medical facilities in Haiti, PIH was involved from the beginning of the epidemic and continues to see an average of 5000 patients per month (April 2011). Two major challenges to the initial crisis were staffing and materials supply chain management. In both cases, partnerships were critical in saving lives in the first days. As cholera had not been previously reported in Haiti, existing healthcare providers had no experience with the rapid fatal nature of the disease and were quickly overwhelmed by volume of patients and severity of illness. Rapid reinforcement of clinical teams with PIH staff from other locations in Haiti provided initial backup but was soon exhausted as the epidemic spread to other regions and staff members were needed at their home base. PIH's partners program in Global Health Equity at Brigham and Women's Hospital, Boston USA sent experienced medical residents for shift work. Technical assistance, particularly in establishing infection control measures was essential for rapid knowledge transfer on best practice and was provided by a partner NGO with cholera experience. Materials and supplies were rapidly consumed. An existing partnership resulted in one NGO in an unaffected area sending materials, supplies and staff within hours of the outbreak without delays of formality. Collaboration with new NGOs in a high-stress environment during a major crisis posed many challenges such as: differing compensation schemes, differing organizational cultures and language skills, duplication of reporting and some degree of competition for resources. Despite these challenges, partnerships were essential to success and should be encouraged by donors, governments and NGOs and established before disasters occur so that response is more efficient during times of crises.

1121

QUALITY OF LIFE IN FILARIAL LYMPHOEDEMA PATIENTS IN COLOMBO, SRI LANKA

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Lymphatic filariasis (LF) is an important global public health and socio-economic problem. It affects 120 million people in over 80 countries, of which, about 14 million suffer from lymphoedema or elephantiasis of legs. Although LF does not cause immediate mortality, the associated severe morbidity has resulted in it being recognized as the second

leading cause of disability worldwide. The two most common chronic manifestations of the disease - hydrocoele and lymphoedema, cause socio-psychological problems to patients and their families. Chronic disease is debilitating, leading to a restriction in the duration and capacity to work and to changes in activity patterns. Secondary bacterial infections of lymphoedematous limbs, known as acute adenolymphangitis (ADL) attacks, contribute to the morbidity of patients as well as progression of the lymphoedema. The quality of life (QOL) was assessed in 141 filarial lymphoedema patients and 128 healthy individuals in the Colombo district of Sri Lanka. Information was gathered by administering the validated translated version of the WHO 100-item QOL questionnaire (WHOQOL-100). This questionnaire ascertains an individual's perception of QOL in the physical, psychological, level of independence, environmental and spiritual domains, as well as the general QOL. There is no documentation of the WHOQOL-100 having been used in filarial lymphoedema patients prior to this study. Healthy controls had a better QOL in all domains as well as in the overall general QOL, when compared to patients with lymphoedema. Several facets such as pain and discomfort, sleep and rest, activities of daily living, dependence on medication and treatment, working capacity and social support were significantly affected by the ADL attack/s patients had suffered. The environmental and spiritual domains were significantly affected by the maximum grade of lymphoedema. The significant difference in the QOL as perceived by patients suffering from filarial lymphoedema and apparently healthy individuals reiterates the importance of morbidity control in patients already affected by filarial lymphoedema.

1122

IMPACT OF A COMMUNITY-BASED LYMPHEDEMA MANAGEMENT PROGRAM ON EPISODES OF ADENOLYMPHANGITIS (ADLA) - ORISSA STATE, INDIA

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India has an estimated 7 million people with lymphedema due to lymphatic filariasis (LF). Clinic-based programs to promote lymphedema management at home have been shown to decrease episodes of adenolymphangitis (ADLA), but the effectiveness of community-based programs, which can potentially achieve higher coverage at lower cost, has not previously been studied. A community-based lymphedema management program was implemented in Orissa State, India in 2007 by the Indian non-governmental organization, Church's Auxiliary for Social Action, in consultation with the Centers for Disease Control and Prevention. Health workers teach lymphedema management techniques to >20,000 lymphedema patients. All 330 lymphedema patients in 20 randomly-chosen villages in areas without a previous lymphedema management program and 45 patients with advanced lymphedema in neighboring villages also without a current program were selected for the study, for a total of 375. Patients were followed over 12 months to evaluate the program's impact. Data were collected at baseline and at 1, 2, 3, 6, and 12 months and analyzed using longitudinal analysis procedures. At baseline, the rate of ADLA episodes per person-month was 0.35 compared to 0.14 at 6 months ($p<0.0001$) and 0.23 at 12 months ($p=0.0047$). The rate ratio (RR) of ADLA episodes per person- month among patients at 6 months compared to baseline was 0.40 (95% CI: 0.50, 0.88) and at 12 months was 0.66 (95% CI: 0.50, 0.88). Significant differences were also seen in the rates at 1 month and 3 months when compared to baseline. A marginal Poisson model showed that the rate of ADLA episodes decreased among patients who wore footwear outdoors (RR=0.66, 95% CI: 0.48, 0.92) and increased among patients who used anti-fungal cream (RR=1.81, 95% CI: 1.15, 2.84). Interdigital fungal infections are a risk factor for ADLA and the increased risk of ADLA episodes among patients using anti-fungal cream is consistent with cream being a marker for this risk factor. These data show a beneficial impact of the program at 12 months, but evaluation at later time points is needed.

1123

FLUBENDAZOLE AS A POTENTIAL MACROFILARIACIDE FOR FIELD USE

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A safe, field-usable chemotherapeutic agent that will rapidly kill adult filarial worms is urgently needed in tropical medicine. Ivermectin, distributed as Mectizan® by Merck & Co.Inc. has had an enormous impact on two major human filarial infections of developing countries, onchocerciasis and lymphatic filariasis. However, a macrofilaricide that safely kills adult filarial worms would be a major contributor to the current efforts to rid the world of filarial infections and the diseases they cause. Given the challenges of discovery and development of agents for human use, a drug as described above is arguably most likely, at least at present, to come from the benzimidazole group of anthelmintics. A field useful agent has typically been required to be administered in an oral dosage form, but a truly safe agent administered by another route, including parenteral approaches, could be acceptable and may even be advantageous. We believe that the most appealing benzimidazole with regard to filarial parasites is flubendazole as it is highly active against filariae in a number of hosts. It has the typical benzimidazole structure with an added fluorine as the major structural difference from other benzimidazoles. Flubendazole is highly efficacious in various experimental filariasis models, including the feline *Brugia pahangi* model, a host in which it occurs naturally. This presentation will review the available data on the use of flubendazole in treating infections with filariae and tissue-residing helminths and describe the characteristics needed for reformulating this important macrofilariacide for potential human use.

1124

REINVIGORATION OF LYMPHATIC FILARIASIS MORBIDITY PROGRAM FOR THE GLOBAL PROGRAM

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The Global Program for the Elimination of Lymphatic Filariasis has two major arms to the efforts, the distribution of anti-microfilarial drugs to break transmission and the morbidity management and disability preventions activities for those already affected by the disease. Despite the good efforts of many institutions and field-working groups, the latter of these two components has often lagged behind the efforts and attention paid to distribution of drugs to the eligible population; this has occurred for many reasons that include poor funding, a general lack of knowledge of the prevalence, and confused understanding of the optimal approaches to treating and assisting these unfortunate people. The actual number of those affected is not known in many countries. There are many reasons why there is great value, in addition to the obvious humanitarian need, in attending to the needs of lymphatic filariasis patients. Attending to patients has a positive effect on improving coverage, and the improvements in patients that has been seen as a result of implementing mass drug administration programs in a number of countries has contributed to improving drug coverage in the whole population. It is vital,with many countries beginning to wind down their drug distribution

programs as infection levels dramatically reduced, that efforts made to attend to those who will still be suffering from the clinical consequences of the infection which extend long after infection and transmission has ceased. These affected people are in danger of being again forgotten when MDA programs cease with elimination. This presentation will review the renewed efforts currently being made to enhance and widen the morbidity management and disability prevention efforts in endemic countries.

1125

THE INTERACTION OF THE INFECTIVE STAGE OF *BRUGIA MALAYI* WITH LANGERHANS' CELLS

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Filarial infections are initiated by deposition of the infective larvae (L3) in the skin, a process that likely conditions the priming of the immune system to the parasite. Thus, understanding the interaction between the L3 and the antigen presenting cells in the skin becomes crucial. Previously, by using ex-vivo epidermal skin explants exposed to L3 in contact or in transwell, it was shown that the expression of IL-18 protein and of mRNA for caspase 1, CD207 (Langerin), and IL-18 binding protein (BP) was induced by the L3. Since caspase 1 is central to the inflammasome, we further investigated the potential involvement of inflammasomes in L3-exposed LCs. We generated human LCs (Langerin+, E-cadherin+, CD1a+) *in vitro* using conventional techniques and exposed them to either L3 (in contact or in transwell), LPS, or media and assessed their expression of cell surface markers, production of pro-inflammatory cytokines and expression of the genes involved in inflammasome activation. In contrast to a known activator of the inflammasome, LPS, L3s only induced minimal up-regulation of surface expressed CD14 (with concomitant down-regulation of CD1a), CD86 and CD83 with no changes in surfaced expressed CD207, E-cadherin, CD80, CD40 and HLA-DR. No significant changes in mRNA expression were seen between LC and LC exposed to L3 for the inflammasome-associated genes NLRPs, NLPR1, NLRC4, AIM2, ASC and IL-18, although there was increased (but not statistically significant) expression of I L-18BP and caspase 1. L3 failed to induce the production of the cytokines IL-1β, IL-6, IL-8, IL-18, IL-18BP, IL-33 and IFN-γ from *in vitro* LCs, nor did the L3 condition the LC to suppress proinflammatory responses to LPS or Poly I:C. The apparent discrepancy between L3 exposed skin explants and the *in vitro* generated LCs can be explained by the presence of keratinocytes (KC) in the ex vivo model; the role of KC/LC interaction in the context of L3 exposure is ongoing.

1126

LOA LOA INFECTIONS AT A TERTIARY REFERRAL CENTER: REFINING THE CLINICAL AND IMMUNOLOGICALLY BASED DIFFERENCES BETWEEN TEMPORARY RESIDENTS AND THOSE INDIGENOUS TO LOA-ENDEMIC AREAS

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Previous studies have suggested that Loa loa infections in inhabitants of Loa-endemic areas (END) have marked differences in clinical presentation compared to those of temporary residents (TR). Many of these differences are thought to be immune-mediated. To assess these differences in clinical outcome and pathogenesis of Loa loa infection, we conducted a retrospective analysis of 181 patients with loiasis seen at the National Institutes of Health. Among the 181, 37 were raised in L. loa-endemic regions while 144 were visitors to these same regions. The initial clinical presentation differed markedly between the two groups with only 41% of END having a history of Calabar swelling compared to 79 % of TR (p<.001). In contrast, the END were much more likely to have had eyeworm (62% compared to 14%, p<.001) and were more likely to have microfilaremia (73% compared to 23%, p<.001). TR were more likely to have an atypical presentation of infection including urticaria (19.4% vs 2.7%; p<.05). There were no differences between the groups in gastrointestinal symptoms (p=.118), neurologic symptoms (p=.29), rashes (p=.33), pruritus (p=.83), or cardiomyopathy (p=.81). Although there was not a statistically significant difference in the serum levels of polyclonal IgE between the two groups (geometric mean [GM] 883 IU/mL in END vs 294.7 IU/mL in TR, p=.651), the serum levels of polyclonal IgG differed significantly (GM 1538 IU/mL in END vs 1168 IU/mL in TR, p<.01). There was no significant difference in filarial (BmA)-specific IgG or IgG4 between the two groups, (BmA-specific IgG 696 mg/mL IgG in END vs 458 mg/mL in TR; BmA-specific IgG4 295 ug/mL END vs 89 ug/mL in TR). Most notably, the absolute eosinophil counts (AEC) were markedly different between the groups; the GM AEC in TR more than two-fold higher (1505/uL compared to 730/uL) than in the END (p=.034). These data extend earlier observations related to immunologically based clinical differences between TR and END. Additional data concerning eosinophil-related pathogenesis of loiasis will be discussed.

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VACCINATION OF BALB/C MICE WITH INTESTINAL ANTIGEN FROM *LITOMOSOIDES SIGMODONTIS* FAILS TO PROTECT AGAINST CHALLENGE INFECTION

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Intestinal antigens have shown promise as vaccine candidates in a number of helminth models. In this study, we evaluated the immune responses that develop towards filarial intestinal antigens in mice infected with *Litomosoides sigmodontis*, a murine model of filariasis in which infective-stage L3 larvae develop into mature adult worms in immunocompetent Balb/c mice. A crude homogenate of soluble intestinal antigens from *L. sigmodontis* worms (GutAg) was prepared from intestinal tracts obtained from adult female worms by microdissection. At both 8 and 16 weeks after infection, splenocyte proliferative responses towards GutAg were substantially lower than that which developed towards a crude homogenate of whole worm antigen (LsAg). Similarly, IgG antibody titers and splenocyte production of both IL-4 and IFNγ were lower in response to GutAg than LsAg at all timepoints studied. Vaccination of mice with three weekly intraperitoneal injections of 10 micrograms of GutAg with CPG and alum as adjuvant resulted in marked splenoctye proliferation and IL-4 and IFNγ production in response to GutAg as well as titers of GutAg-specific IgG antibodies measurable at dilutions up to 1:175. Despite the induction of GutAg-specific immune responses, an initial challenge experiment demonstrated no protection in GutAg vaccinated mice as compared to controls. These preliminary results suggest that filarial infections do not induce large immune responses to intestinal antigens and that inducing such responses may not be protective. Further trials are underway to determine whether vaccination strategies that induce greater antibody titers can be protective.

1128

ENDOTHELIAL CELLS RELEASE SOLUBLE FACTORS THAT PROLONG THE SURVIVAL OF *LITOMOSOIDES SIGMODONTIS* MICROFILARIAE *IN VITRO*

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Microfilariae of most filarial pathogens typically survive for months in their vertebrate hosts. *In vitro*, however, microfilariae live for much shorter time periods. To begin characterizing the factors microfilariae require for

prolonged survival, we have conducted a series of *in vitro* experiments using microfilariae obtained from gerbils infected with Litomosoides sigmodontis, a filarial parasite of rodents. While culture of microfilariae in Dulbecco's Modified Eagle Medium supplemented with 10% FBS resulted in average survival of only 7 days, co-culture of microfilariae with a mouse endothelial cell line (EOMA) extended survival to 40 days. Not all cell lines have this property, as all microfilariae co-cultured with a mouse myeloma cell line died by day 10. Co-culture experiments using EOMA cells in transwell plates extended microfilaria survival as well as direct co-culture, suggesting that the factors microfilariae require are soluble in nature. Heat inactivation of conditioned media from EOMA cells resulted in average microfilaria survival of only 3 days. Together, these findings demonstrate that microfilariae require heat-stable factors released from endothelial cells for prolonged survival. By identifying a cell line that does not promote microfilaria survival, we are poised to begin biochemical and comparative analyses to elucidate the chemical nature of these essential factors. Identification of such factors will advance our ability to cultivate filarial pathogens *in vitro* and may provide insights for the development of new antifilarial compounds.

1129

ENTOMOLOGICAL STUDIES TO RE-EXAMINE THE EVIDENCE FOR MASS DRUG ADMINISTRATION FOR FILARIASIS ELIMINATION IN WEST AFRICAN CAPITALS: THE CASE FOR FREETOWN, SIERRA LEONE

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Anopheles mosquitoes are the principal vectors of lymphatic filariasis in West Africa where the urban mosquito, *Culex quinquefasciatus*, is less susceptible to *Wuchereria bancrofti* found in the sub-region. The transmission of lymphatic filariasis (LF) in West Africa is mostly rural, with little or no evidence of active transmission in the capital cities of Accra, Conakry and Freetown. Night blood surveys carried out on 500 adults in the Western Urban District of Freetown in 2007 revealed no MF positive individuals. However, the district comprising of greater Freetown was considered endemic for LF based on antigen positivity using ICT cards. During the civil war, a large population of Internally Displaced Persons (IDP) moved to Freetown from the rural areas where LF is endemic. A study conducted among these IDPs in 1997 revealed an antigen prevalence rate of 14.5%. Based on the presence of antigen positive individuals in Freetown, MDA was carried out in the capital in 2010. The present study tests the hypothesis that populations of limited low density microfilaremia carriers settling in urban cities in West Africa are incapable of initiating LF transmission by the less efficient *Anopheles* mosquito species. The second objective of the study was to determine the role of *Culex quinquefasciatus* in LF transmission in Freetown. Mosquitoes were collected, using the pyrethrum spray sheet method, from the communities were ICT positive individuals were found. Since May 2009, a total of 6327 *Cx. quinquefasciatus,* 603 *Anopheles* and 6 *Aedes* mosquitoes have been tested by PCR and none was found positive for *W. bancrofti*. These results suggest no evidence for ongoing transmission, and the current MDA campaign in Freetown may not be necessary. This study makes a case for the need to determine active disease transmission for targeted resource utilization, especially since the LF elimination program requires treatment for endemic communities for at least 5 years.

1130

THE CHANGING STATUS OF FILARIASIS IN TANZANIA

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Tanzania has been one of the Africa's leading countries in the global effort to eliminate lymphatic filariasis in Africa. Historically Tanzania has been involved in research for over half a century, and was one of the first countries in Africa to begin the mass drug administration (MDA) of ivermectin and albendazole to eliminate this affliction from the country. The coastal regions of Tanzania has been known for many years to be site were some of the worst prevalences of clinical filariasis in Africa are found with up to 15 percent of adults affected in some way with over clinical signs and symptoms of filariasis. The extent of filariasis in the country was assessed in 1999-2000 as a essential step in developing the national MDA program using rapid antigenaemia tests (ICT) and clinical data of the presence of the disease in each political district in the country. At this time is was found, contrary to what was expected at that time, in fact the whole country was eligible for the initiation of the MDA program for filariasis; thus the target population for the national program was around 35 million people. The MDA program began with a scaling up of new areas each year to reach some 15 million treatments in 2008. Assessment of various districts after 5 and 7 years for MDA has shown that area which were around 70% ICT positive have now been reduced to less than 5% and to around 1% in a number of areas. Analysis of the reduction in prevalence of ICT and circulating microfilarial loads suggests that it is important to consider prevalence levels (e.g. hyper-endemicity versus hypo-endemicity) when considering expectation for the length of time of program implementation, the needs for morbidity control; activities to enhance MDA activities, etc. Data from the archipelago island district of Mafia, with its very heterogenous population (farmers and fishermen) clearly show that adaptation of MDA to suit the local community is essential to obtaining successful reduction on prevalence of the parasite. Tanzania has seen a remarkable change in parasite prevalence and clinical disease as a direct result of the MDA programs.

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QUANTIFYING THE ECONOMIC BENEFITS OF A COMMUNITY-BASED LYMPHEDEMA MANAGEMENT PROGRAM - ORISSA STATE, INDIA

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There are an estimated 59 million people with lymphatic filariasis (LF) in India; an estimated 19.6 million of whom have chronic filarial disease. Orissa State is highly endemic for lymphatic filariasis with many people requiring lymphedema care for lymphedema or elephantiasis. Nevertheless, there are little data on the cost associated with scaling up lymphedema management programs in LF endemic countries. A community-based lymphedema management program was implemented in Orissa State by the Indian non-governmental organization, Church's Auxiliary for Social Action, in consultation with the Centers for Disease Control and Prevention. Over a three year period from 2007-2010, 21,468 lymphedema patients were sequentially recruited into the program in Khurda district, an LF endemic district in Orissa State; 5,478 patients enrolled between 2007-2008, 9,996 patients enrolled between 2008-2009, and 5,994 patients enrolled between 2009-2010. Activities of the program entailed: health education on lymphatic filariasis prevention, disease, and clinical management; lymphedema management training for medical and paramedical staff and community health workers; and support to the lymphedema patients, including provision of soap, antifungal ointment, towels and in some cases footwear. The start-up

cost per patient varied from US $6.75-$9.00 and the maintenance cost per patient was US $3.50. The majority of the total program cost (64%) went to providing direct care (training, follow-up and supplies) for the lymphedema patients. At 12 months after enrollment, patients reported a total of 28.8 (range, 15.6-38.4) fewer lost days of productivity than prior to enrollment in the lymphedema management program. Extrapolated over the entire enrolled population (n=21,468) translates into greater than 1600 person years of productive labor saved over the first year of the program. Despite higher start-up costs, community-based lymphedema management programs can have a broad beneficial impact by improving patient productivity.

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SCREENING FOR NOVEL ANTHELMINTICS THAT ACT ON NEMATODE NEUROPEPTIDE RECEPTORS

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Neuropeptides in the FMRFamide family (FLPs) are essential components of nematode neuromuscular systems and regulate essentially all physiological systems involved in motility, eating and reproduction. A large family of G protein-coupled receptors (GPCRs) that employ FLPs as endogenous ligands have been identified from the free-living nematode Caenorhabditis elegans as well as from many parasitic species. cDNAs encoding ~ 10 FLP-GPCRs have been functionally expressed in yeast (Saccharomyces cerevisiae) in a format that allows facile, multiplexed high-throughput screening assays to identify small molecule, non-peptide ligands that act as agonists or antagonists of these GPCRS. These assays are based on ligand-induced receptor activation, which leads to expression of an enzyme in the histidine biosynthesis pathway that is otherwise absent from this strain of yeast. The presence of an agonist in the culture medium thus permits growth of the recombinant yeast in the absence of histidine, providing a sensitive and highly specific endpoint for screening. Non-peptide agonists and antagonists of nematode FLP receptors are intriguing leads for possible development as novel anthelmintics. We adapted and optimized 3 recombinant yeast strains for screening a collection of synthetic and natural product chemicals held at McGill (HTS/HCS facility, Department of Biochemistry) as part of the Canadian Chemical Biology Network, and for screening collections of diverse natural products and synthetic chemicals in Cape Town and Gaborone. We present here a description of the screening system and results from the initial screening assays of natural products held in collections on both continents.

1133

ANALYSIS OF ß TUBULIN GENE SEQUENCES OF *NECATOR AMERICANUS* IN AREAS OF GHANA WITH LONG-TERM EXPOSURE TO IVERMECTIN FOR ONCHOCERCIASIS CONTROL

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In Kintampo North District (KND), Ghana, ivermectin (macrocyclic lactone) has been used in Mass Drug Administration for Onchocerciasis control since 1980s and recent studies reported albendazole (benzimidazole) failure rates of 39-54% in hookworm treatment. Unlike for human health, benzimidazole (BZ) resistance in the veterinary field is well documented, and is associated with single nucleotide mutations in the β-tubulin isotype-1 gene that result in amino acid changes Phe167Tyr, Glu198Ala and Phe200Tyr in the gene product. Furthermore, it has been reported that the use of macrocyclic lactone could result in such changes. We therefore investigated the sequences of β-tubulin gene for potential BZ resistance marker in Necator americanus obtained from stool specimens of 210 surveyed individuals in rural communities in KND. The prevalence and intensity of hookworm infection was determined using the Kato-Katz method. Hookworm eggs were cultured by a modified Baermann method and DNA extracted from larvae using a modified proteinase K method. Primers for a nested PCR method were designed from published β-tubulin sequences and used together with a proof-reading polymerase to amplify these regions of interest. Eight PCR products were sequenced and aligned using Multalin™. These were translated to amino acids using Expasy™ (Swiss Institute of Bioinformatics). Results indicated egg intensity range of 2688epg - 24epg. *Necator americanus* was the most abundant hookworm with a prevalence of 65% (48/74) while *Ancylostoma duodenale* prevalence was 26.7 % (25/74). Analysis showed several single nuclotide mutations. Nine amino acid changes namely; Phe167Val, Asp197Met, Glu198Lys, Glu198Arg, Thr199Pro, Phe200Ser, Cys201Val, Asp203Ile and Asn204Ile were considered potential resistance markers on the basis that substitution by an amino acid of different properties was likely to affect BZ metabolism. This preliminary study has for the first time, to our knowledge, revealed amino acid changes in the N. americanus β-tubulin gene and we intend to conduct further epidemiologic studies to hone in definitive biomarkers of BZ resistance.

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PARASITIC ZOONOSES CHILDREN UNDER SEVEN YEARS ASSOCIATED WITH THE COEXISTENCE WITH DOMESTIC DOGS

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The objective of the present study was to determine the prevalence of zoonotic parasitic infections in children under seven years, associated with the coexistence with domestic dogs belonging to a village of the township of "Los Garzones" the city of Monteria, Cordoba 2010. The study was descriptive cross-sectional. The sample consisted in 71 families which were selected infant population of 42 children under seven years and all the dogs that lived with the children. After family motivation on work goals and ensure informed consent, we proceeded to the collection of fecal samples in children and dogs. The samples were processed in the Microbiology laboratory of the Faculty of Health Sciences, Department of Bacteriology, University of Córdoba by: fresh preparation, techniques Ritchie method by centrifugation and flotation method, modified Ziehl-Neelsen stain and Graham method. The prevalence of parasitic infections in humans highlighted by *Ascaris lumbricoides* (38.10%), *Strongyloides* spp. (28.57%), hookworm (21.43%). *Entamoeba coli* (33.33%), *Giardia lamblia* (26.19%) and *Cryptosporidium* spp (14.29%). In dogs parasitic infections were found to *Strongyloides* spp. (50.00%), Genera *Ascaris* and *Toxocara*, *Toxascaris* (38.10%), hookworm (33.33%). *Entamoeba coli* (40.48%), *G. lamblia* (26.19%) and *Cryptosporidium* spp (30.85%). In conclusion, the investigation could conclude that in the studied community there are predisposing factors for the submission of parasitic diseases in humans and dogs. The finding of common parasites in children and animals suggest that living with animals is a risk factor for transmission of parasitic infections.

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IDENTIFICATION OF IMMUNDOMINANT *TOXOCARA* EXCRETORY-SECRETORY ANTIGENS

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Toxocariasis is the infection of a human host caused by ingestion of embryonated eggs of the canine or feline roundworm, *Toxocara canis* or *T. cati,* and the subsequent invasion of body tissues by migrating L3 larvae. Infections are divided into 2 major syndromes, visceral toxocariasis and

ocular toxocariasis, both of which can result in extensive tissue damage and, on rare occasions, can lead to eosinophilic meningitis when larvae enter the CNS. Due to widespread endemicity and the risk of morbidity, it is important to have a sensitive and specific assay for diagnosing human toxocariasis. Current diagnosis is dependent on an Enzyme-linked Immunosorbent Assay (EIA) using crude antigen. To develop an improved diagnostic assay for toxocariasis, our aim was to first identify immunoreactive proteins in the *T. canis* excretory-secretory (TES) products from *in vitro* cultivated L3 larvae. Separation of the L3 TES proteins was performed with two-dimensional gel electrophoresis (2DE) on 4-12% Bis-Tris gels. Of three identical 2DE gels, two were transferred to nitrocellulose membranes and probed with either a serum pool prepared from *Toxocara* infected persons or a normal human serum sample; the third gel was stained using a mass spectrometry (MS) compatible silver staining method. Spots showing reactivity when probed with positive sera by Western blot were excised from the silver stained gel for MS analysis. A MASCOT search of the NCBI database identified only 2 *T. canis* proteins from 24 reactive spots suggesting that further studies are needed to define the proteins which could not be identified using existing databases. Choosing the ideal diagnostic protein(s) may require evaluation of up to 24 different antigenic protein targets.

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DIAGNOSIS OF INTESTINAL PARASITIC INFECTIONS USING FLUORESCENCE MICROSCOPY IN CAMEROON

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Intestinal parasites are a real public health problem in developing countries. They are generally responsible for many symptoms among which malabsorption, anemia, abdominal pain. Diagnostic methods based on microscopic identification of parasites remain common in developing countries, despite their low sensitivity. Recently, new fluorescent microscopes with light emitting diodes have improved the diagnosis of other protozoan parasites such as malaria using a DNA-specific dye DAPI (4',6-diamidino-2-phenylindole. This study was designed to compare a rapid fluorescence microscopy - based method for diagnosis of intestinal parasites to classical microscopy and to collect epidemiological data in rural and urban settings to Cameroon. From september 2009 to march 2010, 583 stool samples from outclinic patients were analyzed, including 300 in the city of Douala and 283 in the rural area of Njombe. Each sample was submitted to direct microscopic examination and formalin-ether concentration technique. The observation under fluorescence and white light was made using a fluorescence microscope CyScope® (Partec GmbH, Görlitz, Germany). Stool samples had less visible artifacts under fluorescence and helminth eggs were very clearly observed. In opposite, protozoa were better distinguished using white light. The search for parasites was positive in 155 (26.6%) of the 583 patients in the study. The prevalence in Njombe was significantly higher than Douala (39.2% against 14.7%, P <0.001). The most common prevalent species in Douala was *Entamoeba histolytica* (10.3%), while in Njombe, *Schistosoma mansoni* dominated 13.1%. This work has confirmed a high prevalence of intestinal parasites in a rural area of Cameroon and has also shown that the simultaneous use of white and fluorescence lights for stool exams could help to better observe parasites. Thus, the use of fluorescence microscopy for routine diagnosis of intestinal parasites deserves further investigation.

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MOLECULAR EPIDEMIOLOGY OF ASCARIASIS

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More than 1 billion people are infected with the giant intestinal roundworm, *Ascaris*. Although the greatest numbers of infected individuals are found in Asia and sub-Saharan Africa, ascariasis shows a cosmopolitan distribution and cases are found in both developing and developed countries. We are using molecular epidemiology techniques to study the population structure of *Ascaris* at a global and local scale. Around 550 ascarid worms were obtained from human and pig hosts in East Africa, Asia and Europe. Genomic DNA was extracted from all worms and a 383 base pair region of the mitochondrial cytochrome c oxidase 1 gene (cox1) was sequenced for each worm. Sequences were aligned to identify substitutions, and phylogenetic analysis and assessment of genetic diversity was undertaken. Microsatellite analysis of the *Ascaris* DNA is also underway. Over 70 different cox1 barcodes have been identified in *Ascaris* from humans and pigs so far. There is near complete segregation of barcodes between pig and human worms in Africa but in Europe the same barcodes are found in worms from both hosts. Further analysis should provide insights into the transmission dynamics of *Ascaris* in developed and developing countries.

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STRONGYLOIDES STERCORALIS: METHODS OF DETECTION AND EFFICACY OF TREATMENT IN SCHOOLCHILDREN IN CAMBODIA

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Worldwide, 30-100 millions people are infected with *Strongyloides stercoralis*, one of the most neglected soil-transmitted helminth (STH). Detailed information on the parasite is scare and diagnosis poses a problem. Our study aimed to compare two different diagnostic methods (Koga agar and Baermann technique) for *S. stercoralis* infection in a multiple stool examination approach and to assess the efficacy of ivermectin treatment. We performed a cross-sectional study on *S. stercoralis* infection and STH in 458 children from four primary schools in semi-rural villages close to Phnom Penh by using different diagnostic procedures (Kato-Katz, Koga Agar and Baermann technique) on 3 stool samples. Infected children were treated with ivermectin (200mcg/kg PO, over 2 days) and were reexamined 3 weeks after treatment. Hookworms, *S. stercoralis, Trichuris trichiura* and small trematode eggs (STE) were frequently observed. 24.4% of children were infected with *S. stercoralis*. The sensitivity of Koga-Agar technique and Baermann method was 88.4% and 75.0%, respectively. The negative predictive value of Koga-agar and Baermann was 96.4% and 92.5%, respectively. The cumulative prevalence of *S. stercoralis* was considerably increased from 18.6% to 24.4 after analyzing 3 stool samples by either employed methods, which was much close to the modeled 'true' prevalence of 24.8%. The cure rate of ivermectin was 98.3%. In conclusion, *S. stercoralis* infection is highly prevalent among rural Cambodian schoolchildren. The sensitivity of Koga-Agar technique is higher than Baermann method (88.4% vs. 75.0%). In absence of a "gold standard test", the analyzing of multiple stool samples by different diagnostic methods is required. Ivermectin is highly efficacious against *S. stercoralis* infection and highly cost in Cambodia.

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THE 31 KDA ANTIGEN OF *ANGIOSTRONGYLUS CANTONENSIS* COMPRISES MULTIPLE ANTIGENIC GLYCOPROTEINS

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Human angiostrongyliasis results from accidental infections with intra-arterial nematodes of the genus *Angiostrongylus*. *A. cantonensis* infections result in eosinophilic meningitis and *A. costaricensis* infections cause eosinophilic enteritis. Immunological methods are critical for the diagnosis of both infections since these parasites cannot be isolated from either cerebrospinal fluid or fecal samples. *A. costaricensis* and *A. cantonensis* share common antigenic epitopes which elicit antibodies that recognize proteins present in either species. Detection of antibodies to a 31 kDa *A. cantonensis* protein, present in crude adult worm extracts, is a sensitive and specific method for immunodiagnosis of cerebral angiostrongyliasis. The objective of the present work was to isolate and characterize the 31 kDa protein(s) using soluble protein extracts derived from adult female worms using both single (1DE) and two-dimensional (2DE) gel electrophoresis. Purified proteins were blotted onto nitrocellulose and tested using sera from infected and non-infected controls. The 31 kDa band present in 1DE gels and the 4 spots identified in 2DE gels were excised and analyzed by electrospray ionization mass spectrometry. Four unique immunoreactive proteins with molecular masses close to 31 kDa region were identified based on the highest scores obtained after MASCOT analysis: tropomyosin, the 14-3-3 phosphoserine-binding protein, a nascent polypeptide-associated complex domain, and the putative epsilon subunit of coatomer protein complex isoform 2. Oxidative cleavage of diols using sodium *m*-periodate demonstrated that carbohydrate moieties were essential for the antigenic reaction of all four of the 31 kDa proteins. This data has strong implication for the choice of appropriate vectors to express such recombinant targets for development of diagnostic tests for angiostrongyliasis.

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WHAT CAN FREE-LIVING AND PARASITIC WORMS TELL US ABOUT ANTHELMINTHICS?

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Soil-transmitted helminth (hookworms, *Ascaris* and *Trichuris*) infections are now acknowledged as key contributors to morbidity and poverty worldwide. Although currently there are two approved classes of anthelmintics used to treat human intestinal roundworm parasites, both were initially developed to treat veterinary parasites. We have evaluated *Bacillus thuringiensis* (Bt) crystal (Cry) proteins as novel anthelmintics and considered: 1) how well the effect of a specific anthelmintic on free-living *Caenorhabdits elegans* or the rodent parasite *Heligmosomoides bakeri* might predict efficacy on parasitic roundworms more closely related to those that infect humans; and 2) how the effect of anthelmintics *in vitro* corresponds to that *in vivo*. To address these questions, we have initiated a study of the effects of five different classes of anthelmintics on five different roundworm species, including three in the same genus as human parasites (*Ascaris*, *Trichuris*, *Ancylostoma*) and two not (*Heligmosomoides* and *Caenorhabdits*). We are quantitating the effects of these anthelminthics on viability of all five species *in vitro* and on several *in vivo*. Here we will discuss our work in progress on correlation of anthelmintic effects from roundworm to roundworm, on comparing *in vitro* and *in vivo* results, and on development of Bt Cry protein Cry5B as the novel anthelmintic. We will also discuss the implications of these results for future application of novel anthelmintics for treating human parasitic roundworms.

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CHARACTERIZATION OF ABA-1 EXPRESSION IN EARLY LARVAL STAGES OF *ASCARIS* AND ITS PRESENCE IN HOST FLUIDS IN EARLY AND LATE STAGES OF INFECTION

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Ascaris is a soil transmitted helminth infection that is estimated to affect one sixth of the world's population. Current diagnosis of ascariasis is made using the Kato Katz method of microscopic examination of stool specimens. This is time and labor intensive, varies in sensitivity and specificity depending on the examiner. In addition, diagnosis via stool examination is not possible until one month into the infection when the life cycle is complete. Serological assays have been unreliable because of differences in host responses to Ascaris antigens. The development of an assay that could detect Ascaris antigen in host bodily fluids in the early phase of infection would have wide applicability and utility. Immunoscreening of an *A. suum* infective larval stage 2 cDNA library was performed using sera from infected swine. Nitrocellulose membranes were rinsed, blocked and incubated with primary antibody followed by secondary antibody incubation with anti-pig IgG. BCIP/NBT Sigma Fast was used for staining immune complexes. Only two cDNA clones were strongly recognized by the immune sera. The clones were plaque-purified and their inserts sequenced. These were found to encode different portions of the ABA-1 open reading frame. ABA-1 is an *Ascaris* antigen that has previously been described as a component of the *Ascaris* ES (excretory-secretory) protein which is produced and excreted by all stages of the parasite. Knowledge of the immunodominance of this antigen expressed in early L2 phase of infection will be used to screen timed specimens for the presence of this protein. Recombinant ABA-1 protein obtained from the ABA-1 containing clones will be quantified and used as a control. Mutiple body fluids from infected and control swine will be screened. ABA-1 protein is also expressed by *A. lumbricoides* which infects humans, which makes it an ideal target for use in identifying early infection. The results and their implication for the development of new diagnostic tests of Ascaris infection will also be presented.

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THE THRESHOLD EFFECT: MAGNITUDE AND FREQUENCY OF HOOKWORM LARVAL EXPOSURE DETERMINES THE HOST RESPONSE TO INFECTION

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Hookworm infection affects more than 500 million people worldwide, and represents a major cause of anemia in pregnant women and children. Acquisition of adult worms in the intestine likely results from intermittent exposure to low numbers of infectious larvae via contact with fecally-contaminated soil. Experimental hookworm infection is generally characterized by delivery of a single, relatively large inoculum of third stage larvae (L3) in order to study pathogenesis in a permissive animal model. In an attempt to model the dynamics of naturally-acquired infection more closely, we compared the clinical, immunological and parasitological features of single primary infection (10 L3 vs 100 L3) with twice weekly exposure of hamsters to *Ancylostoma ceylanicum* hookworm larvae. Animals exposed to a multiple high dose (100 L3) larval challenge exhibited similar blood hemoglobin levels, hookworm antigen-specific serum IgG responses, and fecal egg excretion compared to those receiving a single exposure, despite a 20-fold difference in total inoculum. In contrast, animals given repeated low dose (10 L3) exposure had lower blood hemoglobin levels, higher antigen-specific serum IgG responses,

and significantly increased fecal egg excretion compared to their primary challenge counterparts, suggesting continued worm accrual over the course of the 82 day study period. Antigen-specific IgM levels increased throughout the duration of the study in all groups, while IgA antibodies directed at larval proteins peaked in an inoculum-dependent manner 35 days after initial exposure, eventually declining below the detection threshold. These data demonstrate that the frequency and magnitude of hookworm larval exposure influences intensity of infection, pathology, and humoral immune responses to parasite antigens. Furthermore, the data suggest a threshold of exposure below which animals remain susceptible to repeated infection with *A. ceylanicum* hookworms, potentially allowing for more accurate modeling of human infection using a permissive animal host system.

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TRANSCRIPTOME ANALYSIS OF THE ANTERIOR SECRETORY GLANDS OF THE PARASITIC HOOKWORM, *ANCYLOSTOMA CANINUM*

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Hookworms are blood feeding parasitic nematodes that infect almost a billion people around the world. Hookworm disease is characterized by a severe iron and protein-deficiency anemia, malnutrition, and immunosuppression. Currently, there are no FDA approved vaccines for hookworms and deworming chemotherapy does not prevent reinfection. A vaccine is highly desired. The parasitic stage of the hookworm injects potent compounds with immunosuppressive properties, which enable the hookworm to establish chronic infections. The proteinaceous component of the secreted products injected into the host may be the key to a vaccine. However, the identity and origin of many of the secreted proteins are unknown. The purpose of this study was to identify the proteins potentially injected into the host during hookworm parasitism. To identify the proteins expressed in the cephalic and esophageal glands, the head of the parasitic hookworm containing the cephalic and esophageal glands was isolated, and a phage cDNA library was created. In total, 2,350 clones were randomly picked and sequenced using Sanger-based method. The expressed sequence tags (ESTs) were cleaned, clustered, and annotated using dCAS, a semi-automated pipeline for sequence analysis. Functional annotation was added using the BLAST algorithm, and similarity-based searches were performed against various public protein sequence databases. Of the 2,350 clones picked, 1994 were high quality and considered for further analysis. The 1994 ESTs assembled into 673 unique transcripts coding for 511 proteins. The most abundant transcripts expressed in the hookworm head were the excretory/secretory protein 1, predicted to be involved in intracellular trafficking and secretion; the nematode anticoagulant peptide 5, a potent inhibitor of the activated coagulation factor 10 (FXa); platelet inhibitor; the Ancylostoma secreted protein 1, member of the pathogenesis-related protein family; and three unknown proteins with not hits to the NCBI protein database. Of the 673 unique transcripts, 188 had hookworm homologs; the remaining 485 transcripts were novel to the hookworm and their most abundant functional domains were the ShKT toxin domain, with putative potassium channel blocking activity; lectins, putative anticomplement; and many transcripts coding for proteins with unknown function. Future studies will involve functional analysis of abundant transcripts.

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VIRAL LOAD SUPPRESSION IN THE DIRECTLY OBSERVED THERAPY OF HIV SEROPOSITIVE PATIENTS UNDERGOING ANTIRETROVIRAL TREATMENT IN CENTRAL NIGERIA

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Directly observed therapy (DOT) has been identified as a strategy aimed at improving compliance among patients with difficulties adhering to anti retroviral treatment (ART). The rationale for application of DOT in HIV care is based on its successful use in treating non-adherent patients with tuberculosis. The impact of the use of DOT strategy was assessed among 173 HIV sero-positive antiretroviral-naïve patients enrolled for ART at the Jos University Teaching Hospital (JUTH) between March and September 2004. Lamivudine, stavudine and nevirapine combination therapy was administered. Forty six (26.6%) of the patients were placed on daily DOT, 39(22.5%) were on twice weekly DOT, 36 (20.8%) were on once weekly DOT while 52(30.1%) were on self administered therapy. At baseline, the mean weights of the patients were 63.5kg, 59.5kg, 64.3kg and 35.3kg respectively for the various categories. The median CD4+TLC of the various groups were 138cells/µl (range. 10 -356), 138cells/µl (16-334), 100cells/µl, (range 6-340) and 134cells/µl (range 20-362) respectively. The median HIV-1 RNA of the patients were 71,377copies/µl (range 200-3,611,910), 136,302copies/µl (range 200-1,283,250), 186,646copies/µl (range 200-4,472,701) and 149,215copies/µl (range 593-2,675,063) respectively. At the end of 48 weeks, the mean weight of the patients on ART increased to 68.1kg, 68.9kg, 62.1kg and 67.6kg against 63.5kg, 59.5kg, 64.3kg and 62.4kg respectively recorded at baseline in the various categories of treatment. Also the median CD4+ cell counts rose from 138, 138, 100 and 134 cells/ml at baseline in the different categories to 352, 315, 360 and 326 cells/ml respectively at week 48. The viral suppressions (<400copies/ml) among the daily DOT category was 91.9% after 24 weeks and 89.2 at week 48. Among the twice daily DOT group, the suppressions were 74.1% and 85.2% at weeks 24 and 48 respectively. Viral suppressions were 81.3% and 84.4% among the once weekly DOT group after 24 and 48 weeks respectively, while among the self administered therapy group, viral suppressions were 82.1% at week 24 and 79.5% at week 48.

1145

PRENATAL EXPOSURE TO MALARIA ALTERS EXPRESSION OF SELECTED TRANSCRIPTION FACTORS IN CD4+ MEMORY CBMC THAT INCREASES SUSCEPTIBILITY TO HIV *IN VITRO*

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Recurrent or chronic infections in pregnant women living in malaria endemic areas can activate the fetal immune system *in utero* and may increase risk for mother-to-child transmission of HIV. We have previously shown that unstimulated cord blood mononuclear cells (CBMC) primed to malaria blood stage antigens show increased susceptibility to HIV infection *in vitro* compared to CMBC from non malaria primed offspring. To understand the basis for this increased susceptibility of CBMC to HIV infection, we examined the molecular pathways involved in HIV infection of CBMC subpopulations. We found that effector memory $CD4^+$ T cells were the exclusive initial targets of HIV infection, with rapid viral spread to the central memory compartment. Increased expression of CD25 and

HLA-DR was observed on both central and effector memory cells of HIV susceptible vs. not susceptible CBMC indicating ex vivo activation is important in viral susceptibility. This increased susceptibility was not associated with increased viral entry of target cells since detection of minus strand strong-stop DNA twenty-four hours post virus exposure was similar in all samples tested. By contrast *gag/pol* RNA was only detected in HIV susceptible CBMC samples, suggesting that integration or gene transcription of integrated DNA provirus regulates susceptibility. To examine these possibilities we performed a targeted gene expression analysis of the total memory population by PCR array, which reproducibly showed greater expression of *IFNg*, *NFATc1, IRF1, FOS*, and *PPIA* and decreased expression *YY1* and *TFCP2* in HIV susceptible vs. not susceptible CBMC. This suggests that in malaria primed CBMC, activation of host genes that regulate integrated proviral gene transcription increase susceptibility to HIV infection. This system provides a valuable model to understand critical pathways that affect T cell susceptibility for HIV replication *in vivo* and has broader implications that efforts to reduce maternal co-infections during pregnancy may help reduce risk for vertical transmission of HIV.

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PRELIMINARY STUDIES ON HIV-1 ASSOCIATED IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME IN CHINESE HIV INFECTED INDIVIDUALS

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Immune Reconstitution Inflammatory Syndrome (IRIS) is the paradoxical inflammatory syndrome developed soon after antiretroviral therapy initiation. Although many recent studies have reported variable incidence of IRIS, in china however, limited data on clinical and mechanism of IRIS are available. The aim of the present study was to investigate the the immunological and biological factors involved in occurrence in AIDS patients after HAART initiation. 238 AIDS patients who received initial HAART were followed for IRIS over 24 weeks. Clinical manifestations, T-regs, Th1/Th2 cytokines and IL-7 were monitored at base line, onset of IRIS, week 4, 12, 24. RESULTS: IRIS occurred in 47 patients (19.7%) within 28 (9-36) days after HAART initiation. The first case appears only 5 days after HAART initiation and the last case, 150 days later. Systemic OI (OI-IRIS) accounted for (19.7%; 47/238) of IRIS cases, predominantly of Tuberculosis (29 cases), Herpes simplex (8 cases), Herpes zoster (5 cases), Cytomegalovirus (2 cases), Cryptococcal Encephalitis (1 cases). CD4+/CD8+ naive and memory T cells exhibited no significant differences between both groups however, CD4+CD25+Foxp3+ regulatory T cells decreased in IRIS group compared to non-IRIS group. IL-2 and IFN-γ were significantly higher in IRIS group whereas IL-4 and IL-10 were significantly lower in IRIS group. IL-7 decreased gradually during HAART, but was higher in IRIS group during all the follow-up. In conclusion, according to our results, antecedents of opportunistic infections, baseline low CD4 cell count associated to an imbalance of Th1/Th2 cytokine with increased IL-7 may be determinant for IRIS occurrence.

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A UNIQUE INFLAMMATORY PATTERN IN THE BRAINS OF HIV-1 SEROPOSITIVE CHILDREN DYING FROM CEREBRAL MALARIA

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Malaria deaths occur primarily in children in sub-Saharan Africa and are due to severe malaria including cerebral malaria (CM). In Malawi, where the entire population is at risk for malaria, HIV-1 prevalence is 12%. High rates of malaria/HIV coinfection are likely but the effects of HIV on CM pathogenesis and outcome are virtually unknown. Comparing brain pathology of HIV-infected (HIV+) and -uninfected (HIV-) individuals with clinically-defined CM could help identify differences in pathogenesis. The Blantyre Malaria Project (BMP) has found 3 patterns of pathology in children meeting WHO criteria for CM: intravascular parasites alone (CM1), intravascular parasites and parenchymal ring hemorrhages (CM2) and no pathology suggestive of CM (CM3, or faux CM). In this cohort the HIV+ rate is higher among autopsies than in the total cohort (20% vs. 13%) and 57% of autopsies with the CM1 pattern are HIV+ compared to 18% with CM2. Because of the association of HIV with the CM1 pattern we performed immunohistochemistry on brain tissue from a subset of the BMP cohort with clinically-defined CM. These included 10 subjects with the CM1 pattern, 10 with CM2 and 10 with faux CM. Five from each group were HIV+ by antibody-based test. We labeled for HIV-1 p24 and ionized calcium binding adapter molecule 1 (Iba1), a marker expressed in activated microglia and monocytes. No HIV-1 p24 was seen. We found a unique pattern of Iba1+ intravascular monocytes more frequently in HIV+ (8/10) than in HIV- (4/10) subjects. These cells frequently contain hemozoin, appear to completely occupy small vessels and adhere to the walls of larger vessels. In the CM1 group, 5/5 HIV+ and 3/5 HIV- subjects had intravascular Iba1+ cells. In the CM2 group, intravascular Iba1+ cells were seen in 3/5 HIV+ and 1/5 HIV- subjects. There were no intravascular Iba1+ cells seen in the CM3 group regardless of HIV status. We found a unique inflammatory pattern characterized by intravascular monocytes, more frequently seen in HIV+ children dying from CM. Efforts to quantify these cells and further characterize them by other surface markers are ongoing.

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BRAIN ABSCESS DUE TO *SALMONELLA TYPHIMURIUM* AND *MYCOBACTERIUM TUBERCULOSIS* IN A PATIENT WITH AIDS

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The incidence of nontyphoid *Salmonella* infections is common in AIDS patients. However, *Salmonella* infections in the Central Nervous System (CNS) are rare, even amongst HIV positive patients. Tuberculosis is another infection that has reemerged with the advent of AIDS. Ten to 20% of cases of AIDS-related extrapulmonary tuberculosis involve the CNS, but brain abscess due to *Mycobacterium tuberculosis* is rare, with few cases described in literature. We describe a case of a 38 years old man who was diagnosed with HIV in 1999, when he presented with *Toxoplasma* encephalitis. He had an irregular use of the antiretrovirals drugs, and in 2005, he was treated for a tuberculous lymphadenitis, and later a diarrhea and meningitis, both caused by *Salmonella*. In September, four months after the treatment of the *Salmonella* infection, the patient presented with seizures. He was hospitalized and a brain CT scan demonstrated two lesions with contrast ring-enhancement. An empirical treatment for *Toxoplasma* encephalitis, using sulfadiazine, pyrimethamine, and folinic acid, was introduced, but 12 days later the patient had no clinical

improvement and developed mental confusion. The new brain CT scan demonstrated an increase in the size of the lesions. He underwent a brain biopsy draining 15 ml of purulent material. The culture of this secretion was positive *for Salmonella typhimurium* and *Mycobacterium tuberculosis*. The patient was treated for 60 days with ceftriaxone, and specific drugs for tuberculosis were introduced later (rifampicin, isoniazid, pyrazinamide and ethambutol). One year after the diagnosis of brain abscess, the patient still showed residual lesions on CT scan but an important clinical improvement. *Mycobacterium tuberculosis* and *Salmonella* are rare even as an individual etiologic agent of brain abscess and there is no other case in the literature of both microorganisms in the same CNS lesion. In this case we suggested the *Salmonella* treatment maintenance with ciprofloxacin, until immunological improvement was achieved with the use of antiretrovirals.

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MULTIPLE MYELOMA IN A PATIENT WITH AIDS

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Several haematological neoplastic diseases and solid tumors have been associated with HIV infection, such as Kaposi's sarcoma, non-Hodgkin lymphoma (NHL) and cervical cancer. The association of Multiple Myeloma (MM), a malignancy of post-germinal centre B cells, with AIDS has been controversial and there have been found only a few cases described to date. We reported here a 28-year-old HIV-infected woman who was regularly receiving antiretrovirals drugs (tenofovir, lamivudine, efavirenz, atazanavir and ritonavir) and presented a CD4+ lymphocyte count of 155 cells/mm^3 and HIV viral load < 50 copies/mm^3. She was referred in October 2007 for investigation of reduced muscle strength of the right hemibody, and infraclavicular and scalp nodular lesions. CT brain scan showed multiple lytic skull lesions and a soft part density nodule located in the left high convexity parietal, associated with meningeal enhancement. The thoracic radiography showed multiple costal aches lytic lesions. Bone marrow biopsy was normal. Serum protein electrophoresis showed IgG 628 mg/dl (770-1510 mg/dl), IgA 2440 mg/dl (134-297 mg/dl) and IgM 50,7 (67-208 mg/dl) and biopsy of infraclavicular and scalp lesions showed plasmablastic plasmocytoma with clone restriction of lambda light chain. A diagnosis of MM was made and the patient started thalidomide and dexamethasone cycles. After 2 months, the patient presented significant reduction of the lesions and a decrease in IgA levels to 135 mg/dl. In the future, as HIV patients have access to potent antiretrovirals drugs and undergo immune reconstitution, there may be more cases of MM rather than NHL. It would be pertinent to consider MM as part of the differential diagnosis in HIV-associated clinical manifestations and to exclude HIV infection in young patients presenting with myeloma.

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USING *LISTERIA* VECTORS TO OVERCOME HELMINTH INFECTION: GENERATING TH1 VACCINE RESPONSES IN TH2 BIASED, IMMUNE SUPPRESSED HOSTS

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Malaria, TB and HIV remain tremendous disease burdens in much of the world's population and functional vaccines are desperately needed. Although sub-Saharan populations are those that will benefit most from these vaccines, they are also coincident with areas endemic for helminth infection. Infection with one or more species of parasitic helminths may suppress the immune system and has been shown, by our lab and others, to suppress vaccine-specific responses. One goal of our research is to find vaccines that will drive significant vaccine-specific immune responses in helminth infected recipients without the need to eliminate helminth infection prior to vaccination. In the current study, we demonstrate that administration of a *Listeria* vector HIV-1 gag vaccine to mice chronically infected with the helminth parasite *Schistosoma mansoni,* drives significant immune responses to HIV-1 gag CTL and helper epitopes. This observation suggests that *Listeria* vector vaccines are capable of driving vaccine-specific responses in helminth-infected populations. Kinetic studies show the antigen-specific responses are durable and induce CD8+ central memory. Based on these observations, we believe *Listeria* vectors should be considered in the development of new generation HIV-1, malaria or TB vaccines to be administered to populations in sub-Saharan Africa where helminth infection is endemic. Studies are underway to determine if other vectors are also capable of overcoming helminth-induced immune suppression.

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HELMINTH ANTIGENS AS ADJUVANTS FOR HIV-1 VACCINES

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Helminth parasites bias the host immune system towards Th2-type and often induce immune suppression, which has been shown, by our lab and others, to inhibit Th1 vaccine-specific responses. Previous studies have shown the complex mixture of molecules that comprise saline soluble egg antigens (SEA) from *Schistosoma mansoni* function to induce Th2-biasing in naïve individuals. SEA has been used experimentally as Th2-type adjuvant for vaccine antigens. In preliminary studies, we asked if co-administration of SEA with a *Listeria* vector HIV-1 gag vaccine in mice, would suppress host cytotoxic T lymphocyte (CTL) and T helper responses to the HIV-1 gag epitopes. Although co-administration of SEA did bias the host immune system towards Th2-type, unexpectedly, co-administration of SEA with the *Listeria* vector HIV-1 gag vaccine significantly increased the frequency of IFN-γ producing gag-specific T helper and CTL responses over that seen in mice that received only the vaccine. This result suggests that there are components in SEA that are potent inducers of Th1-type responses, which, if identified, could be utilized as adjuvants to promote Th1-type vaccine-specific immune responses for HIV-1 and other vaccines. We are continuing to examine the adjuvant properties of SEA and determine which class(es) of molecules in SEA promotes Th1-type immune responses.

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LOOKING FOR SEX WORKERS IN THE GOLD MINING AREAS OF SURINAME: AN ENUMERATION STUDY

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HIV transmission in Suriname mainly takes place through unprotected sex. Higher HIV prevalence in groups with multiple partners having unprotected sex e.g. Sex Workers (SWs), favor the spread of HIV because of the bridge to the rest of the population. The National Strategic Plan identifies Sex workers as a target group for HIV intervention activities. For advocacy, good planning of intervention activities and the evaluation of HIV program, a valid and reliable estimate of target population is needed. In the last decade small-scale gold mining activities in the interior of Suriname has increased, assumingly leading to a higher influx of SWs to these areas. As part of a size estimation study of Sex workers in Suriname, a rapid ethnographic mapping guided by key informants and gate keepers was done in selected gold mining areas, with a prospective high concentration of SWs. Two localities outside the gold mining areas, with known presence of SWs offering services to gold miners, were also included. Every location was visited by 2 interviewers together with a "resource person" (someone familiar with the population at the site and trusted by them). During a 4 week period, questionnaires were handed out to 192 (189 and 3) consenting SW in the age range of

15 to 49 year. 60% was between 20 - 29 years. Of the SWs surveyed, 58.3% was Brazilian, 28.1% Surinamese, 12% Dominican and 1.6% Columbian. Junior high or lower education level was found in 77.6% of interviewees. 50.4% started sex work at age ≤ 19 year. Looking at "safe sex practices", 78.6% always use a condom and 90% had ever taken an HIV test of which 58.3% in the last year. Covering all areas with high density of people, 192 sex workers were found in the gold mining areas of Suriname. Not included here are the women who primarily do other work (such as cooks, shop keepers etc.), but who according to anecdotal data, also exchange sex for money or goods. From a HIV prevention perspective this is also an important group and additional research is certainly needed.

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TREND IN THE PREVALENCE OF HIV/AIDS IN THE STATE OF MISSISSIPPI: A FIVE YEAR REVIEW

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Acquired immune deficiency syndrome (AIDS) remains a disease of grave concern all over the world caused by the human immunodeficiency virus (HIV). It is one of the dreaded sexually transmitted diseases (STDS) but which can also be spread by contact with infected blood, from mother to child during pregnancy, childbirth, or breast feeding. There is no current cure for it but there are antiviral drugs that can ameliorate its severity. The symptoms vary depending on the phase of infection. Mississippi with a population of 2.9 million is one of the states where HIV/AIDS is most prevalent. The Purpose of this study is to assess the trend in the prevalence of HIV/AIDS in the last five years (2006 - 2010). The study is based on the statistical analysis of the prevalence reports in literature and the Mississippi State Department of Health. The literature review shows that the prevalence of HIV/AIDS in the State of Mississippi in the last five years appears to have plateaued. There is no significant difference on year to year basis from 2006 to 2010 ($P > 0.05$). HIV infection by sex showed a preponderance of males infected as against females (68.4% and 31.6% respectively) for the five years under review. It also shows that reported cases of individuals living with HIV/AIDS by year did not show significant differences ($P > 0.05$). The cumulative cases of HIV/AIDS in the State of Mississippi from 1983 to 2009 are 12,989. Of this number 3,263 (26.1%) is white, 9393 (72.3%) is African American, and 197 (1.5%) is Hispanic. These results are very revealing. The trend shows that the prevalence is high in African Americans. With the exception of a slight decrease in 2010, it appears to be increasing. It is much less in whites and appears to be decreasing. More efforts need to be made to control HIV/AIDS among African Americans.

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COST OF ISONIAZID PREVENTIVE THERAPY WITH AND WITHOUT TUBERCULIN SKIN TESTING AMONG HIV CLINIC PATIENTS IN RIO DE JANEIRO, BRAZIL

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The WHO recommends isoniazid preventive therapy (IPT) for all individuals with HIV once active tuberculosis (TB) is excluded. Brazil is a high TB-burden country whose national guidelines recommend use of tuberculin skin testing (TST) to identify individuals eligible for therapy, but IPT use has been limited. In 2005, the Consortium to Respond Effectively to the AIDS/TB Epidemic began a study (THRio) of IPT in HIV clinics in Rio de Janeiro in order to determine if routine screening for and treatment of latent TB in HIV patients reduces TB incidence in the clinic population. While use of TST and IPT improved under THRio, patients continue to experience significant delay to TST and IPT initiation, which eliminating the TST may reduce. Prevalence and incidence data from THRio and published literature were used to estimate TB incidence, TST, IPT, and HAART coverage in order to determine the effectiveness and incremental program cost of increasing TST and IPT use as well as providing IPT without prior TST. Modeling the expected annual incident TB cases from a hypothetical cohort of 10,000 HIV-positive clinic patients demonstrates that the THRio intervention results in a 6% annual reduction in TB cases with 22 cases averted from baseline, while providing IPT without TST results in a 50% annual reduction, with 176 cases averted. Using costs from published literature and online data, and considering the potential number of cases averted, a cost analysis demonstrates that for a program evaluating 10,000 patients per year, increasing TST coverage to 60% results in an increase in cost of US$38,757.52 in the first year. Conversely, providing IPT without TST to all patients, assuming 75% coverage, decreases annual cost by US$146,127.83 in the first year. Over a five-year period, adjusting for inflation, increasing TST coverage to 60% increases cost by nearly US$200,000, while providing IPT without TST to all patients, assuming 75% coverage, decreases cost by over US$600,000. This analysis suggests that providing IPT to HIV clinic patients without prior TST is the most beneficial strategy regarding number of cases averted and program cost in a high TB burden region.

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PLACENTAL *PLASMODIUM FALCIPARUM* MALARIA INFECTION: FIELD ACCCURACY OF HRP-2 RAPID DIAGNOSTIC TESTS IN AN ENDEMIC SETTING

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It is widely recognized that malaria has a negative effect on the outcome of pregnancy. Pregnant women with little or no pre-existing immunity are at high risk of cerebral malaria, hypoglycemia, pulmonary edema, and severe hemolytic anemia, and fetal and perinatal loss can be as high as 60-70%. However, peripheral blood smear microscopy is not always able to detect malaria parasites due to their sequestration in the placenta. Use of malaria rapid diagnostic tests (RDTs) detecting Histidine Rich Protein-2 antigen (HRP-2) in peripheral blood are a potential alternative. In an endemic setting in Uganda, we compared the accuracy of HRP-2 RDTs to microscopy and placental histopathology in pregnancy. Discordant results samples were spot checked using PCR techniques. Among 434 febrile women tested, 38% had malaria. RDTs had a sensitivity of 96.8% (95% CI 92-98.8), specificity of 73.5% (95% CI 67.8-78.6), a positive predictive value (PPV) of 68.0% (95% CI 61.4-73.9), and negative predictive value (NPV) of 97.5% (95% CI 94.0-99.0) in detecting peripheral *Plasmodium falciparum* malaria during pregnancy. Mosquito net use (OR 2.1) and increasing parity (OR 2.7) were associated with lower risk for malaria. At delivery, RDTs had a 80.9% sensitivity (95% CI 57.4-93.7) and a 87.5% specificity (95%CI 80.9-92.1), PPV of 47.2 (95% CI 30.7-64.2) and NPV of 97.1(95% CI 92.2-99.1) in detecting placental *P. falciparum* infections. At delivery, 25% of peripheral infections were detected by microscopy without concurrent placental infection. Compared to placental histopathology, the combination of RDTs and microscopy improved the sensitivity to 90.5% (95%CI 68.2-98.3) for detecting placental malaria infection and the specificity to 98.4% (95%CI 93.9-99.7). Presence of malaria in pregnancy and active placental malaria infection were 38% and 12% respectively. Use of HRP-2 RDTs to detect malaria in pregnancy was accurate when performed by midwives. A combination of RDTs and microscopy provided the best means of detection placental malaria. With a high sensitivity, RDTs could be an useful tool for assessing Malaria in

pregnancy, further research, including (cost-)effectiveness studies will be needed to assess the potential role off RDTs in malaria in pregnancy control.

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PARASITEMIA INDEX IN PATIENTS INFECTED WITH *PLASMODIUM FALCIPARUM* MALARIA

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Severe *Plasmodium falciparum* malaria known as a medical emergency, the treatment requires institution of intensive care as clinical manifestation of severe *P. falciparum* malaria patients is highly diverse and complex. According to the World Health Organization (WHO) criteria, many factors are utilized for definition of severe malaria. Parasitemia density is an important manifestation for severe malaria determination; however, at present there is no uniform agreement for hyperparasitemia definition to define severe malaria. This study was undertaken to illustrate the clinical manifestations as well as to establish the cutoff point of parasitemia density in *P. falciparum* malaria patients for definition of severe malaria. The presenting clinical manifestations of *P. falciparum* malaria patients were analyzed in relation with parasitemia density. 389 malaria patients, admitted at The Bangkok Hospital for Tropical Diseases, were studied. According to WHO's criteria 2006, 200 cases defined as uncomplicated malaria and 189 cases were severe malaria. Regarding to the statistical methods, it was observed that 1% parasitemia gave the most optimal sensitivity and specificity of 79.3 and 73.5, respectively with accuracy of 76.3%. In addition, we found that 1 % parasitemia revealed a statistically significant association with disease severity, low platelet counts, increasing of blood urea nitrogen and creatinine, increased of serum transaminases, jaundice, pulmonary edema, metabolic acidosis, prostration and schizontemia. In conclusion, presenting syndromes of severe *falciparum* malaria depend on many factors. For hyperparasitemia definition, 1% of parasitemia infected red blood cell could be considered as a cutoff point for severity definition, particularly in low transmission area.

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HOW MUCH REDUCTION IN THE OVER-DIAGNOSIS OF MALARIA CAN BE EXPECTED WITH THE USE OF TESTS IN RURAL GHANA?

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To reduce the over-diagnosis of malaria, WHO now recommends that diagnosis be confirmed by tests in all transmission settings. We conducted a cross-sectional study in a district hospital in rural Ghana (from January 2009 to February 2010) to assess the extent of over-diagnosis of malaria and how much reduction could potentially be achieved with the use of rapid diagnostic test (RDT) or microscopy. Under-five children presenting with a history of fever were managed presumptively while samples were taken for malaria RDT and smear microscopy. A total of 936 children were enrolled: 775 in the wet season and 161 in the dry season. Overall 689 (73.6%) were presumptively diagnosed with malaria. Had diagnosis been based on rapid diagnostic test or microscopy, 618 (66.0%) and 404 (43.2%) cases respectively would have been diagnosed with malaria. Using RDT in the wet and dry seasons, reduction in malaria diagnosis would have been 4.1% and 24.2% (diff 20.1%, CI 13.3% - 26.9%, p<0.001) respectively. With microscopy, the reduction would have been 30.5% and 29.8% (0.7%, CI -7.1% - 8.5%, p=0.86) respectively. Using microscopy as standard, the sensitivity and specificity of the RDT used were 97.7% and 58.1% respectively. The anticipated reduction in malaria over-diagnosis may be limited by the low specificity of RDTs and their cost-effectiveness is likely to be season-dependent.

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PERSISTENCE OF *PLASMODIUM OVALE CURTISI, P. OVALE WALLIKERI* AND *P, MALARIAE* IN ASYMPTOMATIC GHANAIAN SCHOOL CHILDREN TREATED WITH DHA-PIPERAQUINE

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Microscopy remains the gold standard for the diagnosis of malaria in the field despite its limitations. However, there remains an under estimation of the true malaria burden especially of less prevalent and less documented species such as *Plasmodium ovale* sp. and *P. malariae*, both of which frequently exist as low density infections. We used standard species-specific nested polymerase chain reaction, targeting the small subunit ribosomal RNA gene, to investigate the presence of malaria parasites in a total of 274 filter paper blood spots from asymptomatic school children in Pokukrom in the Ahafo Ano South district, Ashanti region, in the Southern zone of Ghana. One hundred and forty-five pupils who were microscopically positive for *P. falciparum* asexual parasitaemia were subsequently treated with dihydroartemisinin piperaquine and followed up for 28 days. Of the 274 pre-enrolment samples analyzed, 210 (77%) were positive for *P. falciparum*. Many of the infections were shown by PCR to be comprised of multiple species with 44 (16%) also harbouring *P. ovale* sp., and 77 (28%) harbouring *P. malariae*. There was no evidence of *P. vivax* in our study participants. *P. ovale* positive samples were further classified into *P.o.curtisi* and *P.o.wallikeri* by nested PCR at two different loci (Plasmodium ovale tryptophan-rich antigen (Potra) and Plasmodium ovale glyceraldehydes-3-phosphatase (Pog3p) followed by sequence analysis. In a small number of cases, recurrent parasites were detected by species-specific PCR 28 days after treatment. All three species were represented, unexpectedly, with cases of ovale (4 of 44) and malariae (3 of 77) recurrence, confirmed by PCR. This is the first report of recurrence of these species within 28 days of ACT treatment. There is an urgent need to improve diagnosis of these overlooked non-*falciparum* malaria parasites and to determine their *in vivo* sensitivity to currently used antimalarial drugs. *P. ovale* sp. and *P. malariae* are common throughout sub-Saharan Africa, and thus are important targets for malaria control and elimination.

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SUPERVISORY VISITS IN BENIN SHOW IMPROVEMENTS IN MALARIA MICROSCOPY

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Researchers have suggested that, "supervision and audit with feedback is generally effective," in achieving and maintaining high-quality performance of health workers in low-resource settings (published reports). In FY2010 IMaD conducted regular, quarterly visits to 60 health facilities in Benin. Due to the staggered approach taken by IMaD for scaling up the number of facilities, only 36 of the 60 health facilities were visited a total of four times by the end of FY2010. During each round of supervisory visits IMaD supervisors, who were previously trained in health facility evaluations and evaluated for malaria microscopy competency, collected information on the current state of health facility practices pertaining to malaria diagnostics and treatment. During these visits, supervisors also provided on-the-job training for individual staff members where deficiencies in their performance in conducting routine

diagnostic procedures (e.g., slide preparation, staining and reading), general laboratory practices (e.g., record keeping, inventory, QA/QC) or treatment of malaria (e.g., discussion of proper treatment) were detected. Supervisors provided comments and feedback to health facility staff during each visit, and suggested methods of improving specific practices when necessary. Maintaining continuity with respect to the cadre of supervisors routinely conducting the visits ensures accountability among the health facility staff to improve performance while concurrently fostering a reliable system of support. Over the course of four visits the percentage of health facilities performing microscopy in full consistency with national guidelines increased from 58.2% to 100% by the fourth of the FY2010 visits. Another figure suggestive of the positive impact is the decrease of antimalarial prescriptions to individuals with negative malaria results, which is, alternatively, a measure of prescriber adherence to malaria laboratory tests. The percentage of health facilities that prescribed antimalarials to negative patients fell from 73.1% to 40% by the end of the FY2010 supervisory visits.

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COMPETENCY AND PROFICIENCY ASSESSMENT CAN IMPROVE PARASITE DETECTION AND SPECIES IDENTIFICATION

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Malaria is the leading course of morbidity and mortality in sub-Saharan Africa responsible for 90% of annual global burden. Accurate diagnosis of malaria is important to ensure correct case management. Studies have shown that microscopy in field conditions has a sensitivity of 68.6% and specificity of 61.5%. AMREF and WHO AFRO introduced a competency assessment course for malaria microscopists based on the model approved by the WHO to assess microscopists. Methodology: A five day training course comprising theoretical lectures and laboratory practical sessions were developed based on the WHO recommendations. Well characterized slides sets were used. Pre and post course practical evaluations consisted of 16 and 55 slides respectively. Twenty slides were negative, ten contained *Plasmodium falciparum* with parasite density range 80-200 parasites/mL, and ten slides had *P. malariae*, *P. vivax* and mixed parasite species. Fifteen slides containing *P. falciparum* were used to assess parasite quantification. Results: Eighty five microscopists have participated from 15 countries. Overall, species identification marginally improved from 51.3% - 71.7% (mean 20.4%, 95% CI 04-41; p= 050). Sensitivity significantly improved from 57% - 91% (mean 34%, 95% CI 17-50%; p=0.003), while specificity improved from 62.2- 90.7% (mean 28.5% 95% CI 10-41%; p=010). Parasite quantification improved from 28.3 - 41.7% (mean 13% 95% CI 8-18%; p<0.00.1). Conclusion: The data shows that participation in proficiency testing programmes can improve performance in malaria parasite detection and species identification. There is a need to translate training materials into French and Portuguese to expand the training in Africa.

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ADHERENCE OF HEALTH CARE WORKERS TO MALARIA RAPID DIAGNOSTIC TESTS IN FEVER PATIENTS ATTENDING PRIMARY HEALTH CARE FACILITIES IN ZANZIBAR

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Zanzibar has recently undergone a rapid transition from high to low malaria transmission. In the new epidemiological context it is critical to target malaria treatment, i.e. artemisinin-based combination therapy (ACT), to patients with confirmed malaria infection. To improve fever case management Zanzibar has introduced malaria rapid diagnostic tests (RDT) in all public health care facilities. This study aimed to evaluate health care workers' adherence to RDT in Zanzibar. The study was conducted in 12 public health facilities, 6 each in North A and Micheweni districts. Prior to the study start all health workers were trained in the recently adapted integrated management of childhood illness (IMCI) guidelines as well as standard malaria treatment guidelines. We enrolled 3893 patients, 1824 5 years of age with fever or history of fever in the preceding 24 hours between May and August 2010. All patients were tested with RDT. Overall 122 (3.1%) patients were RDT positive, of whom 38 were <5 and 86 >5 years of age. Among the 3771 RDT negative patients only 2 (both >5 years) were prescribed ACT. Some 121 of 122 RDT positive patients received treatment with antimalarial drugs, 116 with ACT, 4 with quinine and the remaining patient with ACT and quinine. In conclusion, adherence to RDTs results among health care workers in Zanzibar was excellent in the new epidemiological context with low malaria transmission.

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RAPID DIAGNOSTIC TESTS IN THE CAMBODIAN PRIVATE SECTOR: HOW (WELL) ARE THEY BEING USED IN PRACTICE?

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Access to good quality Artemisinin-based Combination Therapy (ACTs) has recently been given a boost with the launch of the Affordable Medicines Facility malaria (AMFM) pilot, which supports a manufacturer level subsidy for the provision of ACTs through public, non-governmental organisation and private sector channels. In the mean time, there has been a dramatic decrease in malaria in many malaria-endemic countries, increasing the need for better targeting of the drugs. The increasing availability of cheap, reliable malaria Rapid Diagnostic Tests (RDTs) means that serious consideration is now being given to the use RDTs outside of public health facilities, including the private sector. However programmatic experience of RDT in this sector is limited. In 2004 Cambodia became the first country to implement a nationwide programme of subsidised and socially market malaria Rapid Diagnostic Tests (RDTs). A combination *Plasmodium falciparum*/non-*falciparum* test is currently sold from Population Sciences International (PSI) to wholesalers and retailers for $0.50 for a box of ten, allowing for a substantial profit. However, little is known about how the RDTs have actually been used in practice. In late 2010 we carried out a drug outlet survey, RDT user assessment, RDT quality assessment and mystery client study in order to document current practice and quality. Over half of the 217 providers interviewed sold RDTs, the vast majority being the socially marketed brand. They were generally stored in adequate conditions and quality of the tested RDTs was good. Providers appeared to be aware of the need for blood testing and reported few problems although observation of their use suggested there was some

areas in which improvements could be made including time-keeping and safe disposal. We discuss the implications of the findings for future implementation in Cambodia and beyond.

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COST-EFFECTIVENESS ANALYSIS OF INTRODUCING RAPID DIAGNOSTIC TESTS (RDTS) FOR MALARIA DIAGNOSIS IN PUBLIC HEALTH CENTERS WHERE MICROSCOPY IS AVAILABLE AND PERIPHERAL CLINICS WHERE ONLY CLINICAL DIAGNOSIS IS AVAILABLE: THE CASE OF GHANA

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Overdiagnosis of malaria is a problem in most parts of Africa. Current evidence in Ghana suggests that overprescription of antimalarials occurs in public health facilities both where microscopy is available and where diagnosis is done presumptively due to lack of parasitological testing facilities. Introducing rapid diagnostic tests (RDTs) for malaria in public health facilities in Ghana may potentially improve diagnosis and may therefore also be a cost-effective intervention. This study was designed to assess the cost-effectiveness of introduction of RDTs in three public health facilities in Dangme West district of Ghana. Suspected malaria patients attending a health facility with a functioning microscope were randomly assigned to diagnosis by either an RDT or microscopy and subsequent treatment by health centre staff whereas suspected malaria patients visiting two other health centres without microscopy were randomly assigned to diagnosis by an RDT or presumptive diagnosis based on clinical signs. Costs of offering diagnostic services and outpatient services were collected through visits to the health facilities. An exit survey among patients with suspected malaria was used to capture and subsequently cost the drugs prescribed irrespective of final diagnosis. Patients were followed up two weeks later in their homes to inquire about any additional health care seeking since the first visit and the associated household costs. The measure of effect was the number of correctly treated patients by diagnostic arm as determined by a double read blood slide. Among the suspected malaria patients visiting the health facility where a microscope was available, it was found that the proportion of correctly treated patients was similar between the RDT and the microscopy arms and that the costs per correctly treated patient were at a similar level. In the two health centres with no microscope, the proportion of correctly treated patients was higher and the costs lower in the RDT arm as compared to the clinical diagnosis arm.

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THE BENEFITS AND PITFALLS OF EX VIVO AND *IN VITRO* SUSCEPTIBILITY TESTING OF *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES

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Drug susceptibility testing of *Plasmodium falciparum* isolates has not been established to the standard currently used in bacteriology, virology, or mycology. No breakpoints have been determined for the interpretation of IC_{50} susceptibility testing results. Different testing modalities exist and are challenged by time-consuming and laborious culture adaptation procedures. One possible solution has been ex vivo testing of patient blood without culture adaptation. We conducted a three year long effort to establish antimalarial drug susceptibility testing of clinical isolates in Toronto, Canada in returning travelers. Testing was conducted using both the SYBR green *in vitro* and HRP ex vivo methods. IC_{50} data for chloroquine, mefloquine, atovaquone, and artemisinin derivatives were obtained for clinical isolates and compared to reference strains 3D7 and W2. Isolates were also sequenced for single nucleotide polymorphisms previously linked to antimalarial resistance. Our results demonstrate that in the main IC_{50} data from ex vivo and *in vitro* methods do correlate well. However, in certain instances polyclonal infections can confound testing results where selection for a fit parasite clone affects the outcome of both IC_{50} and SNP genotyping. The concomitant testing of control strains 3D7 and W2 enable IC_{50} data to be presented as a ratio. This is important as the IC_{50} result can vary by run and method. Exchange of strain panels between reference laboratories is also essential to the maintenance of quality assurance. We conclude that a set panel of strains and isolates be established for quality assurance purposes and that efforts be augmented to correlate IC_{50} data with clinical outcomes in order to establish clinical breakpoints.

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KINETICS OF PARASITE CLEARANCE TIME BY QUANTITATIVE REAL-TIME PCR AND MICROSCOPY IN SUBJECTS WITH UNCOMPLICATED *FALCIPARUM* MALARIA

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Malaria microscopy, performed on Giemsa stained thick and thin smears, has long been the accepted gold standard for detection of parasites in both the clinic and the field. However, we hypothesized that species-specific real time (RT) PCR would enhance existing methods of parasite detection to confirm low-level parasitemia in settings such as clinical trials where they could be of clinical relevance in predicting outcomes. In a randomized, open label clinical trial conducted in western Cambodia, we assessed a species-specific 18s rRNA genomic DNA RT-PCR assay developed in-house, and compared results with expert microscopy. One hundred forty three subjects with uncomplicated *P. falciparum* malaria were randomized to receive 1 of 3 artesunate monotherapy regimens. Blood for microscopy and RT-PCR was collected pre-treatment, at 2, 4, 6, 8, 12, 18 and 24 hours after the first dose, then every 6 hours until 2 successive slides were negative by microscopy, then daily until discharge on Day 6, and then weekly until Day 42. Geometric mean (95% CI) parasite clearance times were 69.7 (64.8-74.9), 88.8 (79.1-99.6) and 150.5 (130.8-173.2) hours for microscopy, by genus specific RT-PCR, and by species-specific RT-PCR respectively (p=0.0001). The percentage of subjects remaining parasitemic at 72 hours after treatment began was 51, 73 and 89% for microscopy, genus specific RT-PCR and by species-specific RT-PCR, respectively. In most subjects who subsequently failed treatment, both qRT-PCR and microscopy became negative before the day of failure. However, among the failures, several were positive for malaria earlier by qRT-PCR than by microscopy. These data suggest that determination of parasitemia at 72 hours by qRT-PCR is a more sensitive indicator of parasite clearance than microscopy, and that, in spite of the increased sensitivity recrudescent parasites still fall below the limit of detection for this assay. However, this argues for a role of qRT-PCR in clinical trials of antimalarial therapy, in addition to more traditional clinical endpoints.

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IN VITRO METABOLISM-LINKED HEMOTOXICITY ASSAY: VALIDATION AND APPLICATION OF THE ASSAY TO SCREEN NEW ANALOGS AND UNDERSTAND THE MECHANISM OF HEMOLYTIC TOXICITY OF 8-AMINOQUINOLINE ANTIPARASITICS

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Metabolites generated through cytochrome P_{450}-dependent metabolic reactions are responsible for hemolytic effects of primaquine (PQ) and other 8-aminoquinolines (8-AQs). The hemotoxic response of the metabolites generated *in situ* could be measured by estimation of accumulation of methemoglobin (mtHb), kinetic measurement of increase in oxidative stress, and depletion of reduced glutathione (GSH) in a microsomal metabolism-linked hemotoxicity assay (Ganesan *et al*, Toxicol Appl Pharmacol. 2009; 241:14-22). The assay was validated with two blinded sets of non-hemolytic and hemolytic drugs. Twelve of twelve clinically reported non-hemolytic drugs tested negative, and eight of nine hemolytic drugs tested positive in this assay, the exception being acetanilide. 8-AQ analogs have also been evaluated. Several agents that replenish intracellular reduced thiols and/or protect the cells from oxidant injury were tested for mitigation of hemotoxic effects of PQ metabolites. N-acetyl cysteine (NAC) has been reported to produce an increase in intracellular GSH, and decrease in oxidative stress. NAC partially attenuatee the hemotoxic effects of 5-hydroxyprimaquine (5-HPQ), a potential hemotoxic metabolite. A comparative evaluation of 5-HPQ and 8-N-hydroxy-6-methoxy-aminoquinoline (MAQ) showed differential hemotoxic responses. 5-HPQ produced about a 3- fold higher mtHb and more prominent depletion of GSH in G6PD-deficient human RBCs than MAQ; however, MAQ generated about 3-fold higher oxidative stress than 5-HPQ. In view of the structural similarities and oxidant potential of aminophenols (APs) and hydroxylated metabolites of 8-AQs, several AP analogs were evaluated *in vitro* for their hemolytic effects. The 2-APs generated markedly higher hemotoxic response compared to 4-APs, but 3-APs were non-toxic. 4-Methyl and 4-chloro substitutions potentiated the toxicity, while 4- and 5-nitro substitutions completely attenuated the toxicity of 2-APs. The results suggest possible structure-toxicity-relationships of APs and may be useful in designing new non-hemolytic 8-AQ analogs.

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PHENOTYPING PRIMAQUINE METABOLITES *IN VITRO* BY PRIMARY HUMAN HEPATOCYTES USING UPLC-QTOF-MS WITH STABLE ISOTOPE LABELING

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Primaquine (PQ) is an important antimalarial agent because of its activity against exoerythrocytic forms of *Plasmodium* spp. However, hemolytic anemia is a dose-limiting side effect of primaquine therapy that limits its widespread use in the clinic. The hemotoxicity is believed to be mediated by metabolites; however, the identity of the toxic species has remained unclear due to their highly reactive nature. The major plasma metabolite identified in humans and animals, carboxyprimaquine (cPQ), appears not to be responsible for this toxicity. Identification of minor metabolites in biological matrices poses a major challenge. Drug candidates labeled with stable isotopes in combination with LC/MS can be used to overcome this problem. This study was undertaken to identify the metabolites using UPLC-QTOF-MS from *in vitro* incubation of a 1:1 w/w mixture of $^{13}C_6$-PQ/PQ with primary human hepatocytes. An Acquity UPLC™ BEH Shield RP18 column (100 mm × 2.1 mm I.D., 1.7 µm) was used. The mobile phase consisted of water and acetonitrile, both containing formic acid at a flow rate of 0.25 mL/min with gradient elution. Acquity UPLC was integrated with QTOF-MS to combine the efficiency of separation with high sensitivity, selectivity of detection, and accurate mass. Qualitative metabolite identification was performed using MetaboLynx XS software. The lock mass compound was leucine enkephalin (*m/z* at 556.2771 and 278.1141). UPLC retention time, twin mass peaks with difference of 6 (originating from $^{13}C_6$-PQ/PQ), MS/MS fragmentation pattern, and percentage of metabolite (relative area% with respect to parent compound) were used for phenotyping and semi-quantitative analysis of metabolites. Besides cPQ, formed by oxidative deamination to aldehyde and subsequent oxidation, several other metabolites were identified: including PQ alcohol from oxidative deamination to aldehyde and subsequent reduction, the alcohol glucuronide conjugate and its acetate, as well as trace amounts of quinone-imine metabolites of PQ and cPQ, perhaps from hydroxylation at 5-position and subsequent oxidation.

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ANTIMALARIAL ACTIVITY OF METHYL JASMONATE AND EFFECT ON LIPID PROFILE OF *PLASMODIUM BERGHEI* INFECTED MICE

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Efforts at eradicating malaria has not yielded the desired results due to various challenges part of which is due to parasite resistance to commonly used antimalarial drugs. As part of the search for new antimalarial drugs, we screened methyl jasmonate (MJ), a fatty acid derived cyclopentanone and a component of the essential oil from flowers of *Jasminium grandiflorum* for *in vivo* activity in mice. *In vitro* study had indicated potential antimalarial activity of MJ. The Rane test procedure was used to assess the antimalarial activity of MJ. Forty-two BALB/C mice were infected with *P. berghei* NK65 (1 x 10^7) and divided into 6 groups. Groups 1, 2, and 3 received 10, 25 and 50mg/kg body weight of MJ respectively.

Groups 4, 5, 6 and 7 received chloroquine 10mg/kg, arteeether 3.2mg/kg, ethanol and normal saline respectively. All treatments were administered daily orally for four consecutive days. Thick and thin blood films were made from each mouse for 7 days and weekly for 28 days, stained with Giemsa stain and examined microscopically for parasitaemia. Twenty four hours after last administration, 3 mice from each group were sacrificed with serum used for liver function test and cholesterol, triglyceride, HDL and LDL determinations. Mean survival time were also documented. Methyl Jasmonates treatment resulted in a dose-dependent reduction in percentage parasitemia relative to control. 50mg/kg of MJ caused 54.4 % decrease in parasitaemia relative to chloroquine 81.3% and arteether 99.5% by Day 3. Mean survival time for 50mg MJ was 22.6 days compared with untreated (10-5 days), chloroquine (31.5 days) and arteether (27.2days). MJ like chloroquine and arteether treatment caused a marked decrease in cholesterol, triglyeride and HDL relative to untreated infected mice. There was a significant decrease in alkaline phosphatase MJ caused significant reduction in parasitaemia in a dose-dependent manner but less effective than chloroquine and arteether. MJ did not affect liver function enzymes and lipid profile adversely.

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NOVEL ANTI-MALARIALS: NAPTHOTHIAZOLIUM SALTS WITH POTENT ACTIVITY AGAINST *PLASMODIUM FALCIPARUM IN VITRO* AND *P. BERGHEI IN VIVO*

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Because of emerging resistance to existing drugs, novel classes of anti-malarial drugs with new mechanisms of action are needed. A large library of compounds was synthesized and designed to accumulate in the digestive vacuole of the malaria parasite and potentially catalyze the breakdown of hemozoin. Eight compounds in the original library were highly active against *Plasmodium falciparum in vitro*. The two most promising compounds are amphiphillic naphthothiazolium salts with amine-bearing side-chains. The most active compounds identified thus far are (1) KSWI-19855 which has an IC_{50} of 75nM against both chloroquine-sensitive and chloroquine-resistant *P. falciparum* (strains D10 and Dd2) and (2) KSWI-19854 which has an IC_{50} of 75nM against chloroquine sensitive *P. falciparum* (strain D10) and 0.5μM against chloroquine resistant *P. falciparum* (strain Dd2). In murine *in vivo* efficacy studies, both KSWI-19854 and KSWI 19855 demonstrate greater than 90% activity against *P. berghei* at 10mg/kg/day for 4 days. We postulate that these amphiphilic compounds reversibly enter the lipid nanospheres where hemozoin is synthesized inside the parasite food vacuole. Once in the food vacuole, we postulate that they depolymerize hemozoin by reducing the Fe^{+3} in hemozoin to its Fe^{+2} oxidation state, thereby breaking the iron carboxylate bonds holding the crystal structure together. Dose ranging studies and studies on the mechanism of action are on going. This project may lead to the clinical development of a desperately needed new anti-malarial drug.

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DEVELOPMENT OF SECOND GENERATION REVERSED CHLOROQUINE DRUGS

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Drug resistance is now seen against all of the approved antimalarial drugs. While eradication is the ultimate goal, currently there is still a need for new therapies to help those afflicted with this disease. We have previously reported on our 'Reversed Chloroquine' (RCQ) compounds, of which our lead candidate is undergoing preclinical testing. Here we present a structure-activity relationship (SAR) study designed to develop a 'second generation' candidate, to be ready in the event our primary drug stumbles on the preclinical road. Specifically, these next-generation RCQ molecules are designed to continue to improve the toxicity profile, while maintaining excellent *in vitro* and *in vivo* antimalarial activity.

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ANTIMALARIAL ACTIVITY AND TOXICITY OF 5 AND 7-METHYLATED PRIMAQUINE ANALOGS

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Primaquine, an 8-aminoquinoline derivative, is the drug of choice for radical cure of relapsing malaria caused by *Plasmodium vivax*, and is also used as a causal prophylactic agent against both *P. vivax* and *P. falciparum*. Primaquine in combination with clindamycin has also been shown to be effective for prophylaxis and treatment of *Pneumocystis carinii* pneumonia in AIDS Patients. A serious limitation to widespread use of this class of drugs, however, is that they produce reversible methemoglobinemia and hemolysis in individuals who suffer from hereditary glucose-6-phosphate dehydrogenase deficiency. Imino-quinone formed by oxidation of the 5- or 7-hydroxylated primaquine metabolite has been postulated to be responsible for this toxicity. If this mechanism is indeed involved, then substitution of a methyl group at 5 and/or 7- position in the quinoline ring of PQ can block the formation of the toxic metabolites. We prepared 5-, or 7-methylated, 5,7-dimethylated as well as 5-methoxy-7-methylprimaquine analogs and evaluated them for *in vivo* antimalarial activity in *P. berghei* mouse malaria model and *in vitro* methemoglobin formation in red cells incubated with the compounds in the presence of pooled human liver microsomes. Methyl substitutions at the 5 or 7 positions dramatically reduced the toxicity, but these analogs were also devoid of antimalarial efficacy. However, introduction of a methoxy group at the 5- position of primaquine improved the antimalarial activity but also increased its methemoglobin generating capacity in the *in vitro* assay. Introduction of 7-methyl group to 5-methoxyprimaquine greatly reduced both activity and toxicity. These results suggest that the blocking of activation of the 5 and 7 positions of the quinoline ring by methylation significantly reduces both toxicity and activity. These results will be discussed in light of the impact of other structural modifications that may improve the therapeutic window.

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LEAD OPTIMIZATION OF LIVER STAGE ACTIVE ACRIDONE ANTIMALARIAL

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Drugs targeting liver stage malaria offer many advantages in the prevention and eradication of the disease, but nearly all of the antimalarials currently in use or under development primarily act on blood stage infection. We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced *Plasmodium* infection in addition to efficacy against the blood stage malaria. Significant improvement was achieved in the lead optimization process, and our latest lead candidate demonstrates potent efficacy in the following system: a) Prevention of *in vitro Plasmodium*

berghei sporozoite-induced development in human hepatocytes with an IC_{50} value of 2.2 ng/ml, comparable to that of atovaquone; b) Full protection from *in vivo P. berghei* sporozoite-induced liver stage infection in mice at 40 mg/kg/d (3X, oral doses); c) Low nanomolar inhibition of *in vitro P. falciparum* blood stage growth against a panel of multidrug resistant parasites; and d) Curative efficacy after oral administration against patent infection with *P. yoelii* in an erythrocytic murine model with an ED_{50} value of 1.2 mg/kg/d (3X), superior to chloroquine in the parallel study. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

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EARLY-STAGE PRECLINICAL DEVELOPMENT OF REVERSED CHLOROQUINE (RCQ) HYBRID DRUGS

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We previously disclosed a class of molecules, termed Reversed Chloroquine compounds (RCQs), comprising a chloroquine (CQ)-like moiety linked to a Reversal Agent (RA) moiety, which reverses chloroquine resistance (CQR) in malaria. Structure-activity relationship (SAR) work has shown that the RCQ design is very flexible. We have constructed a substantial library of RCQ molecules that display *in vitro* efficacy - even sub-nanomolar IC50 values - against both CQR and CQS *Plasmodium falciparum*. The RCQ molecules have enhanced uptake, relative to CQ, into CQR parasites; they also diminish the activity of CQR-associated PfCRT protein mutants which have the ability to enhance efflux from the parasite's digestive vacuole. A subset of these drug candidates has been tested in mouse models of malaria, and found to be capable of reducing the parasite burden to below detectable limits - an oral cure. Both cytotoxicity and acute toxicity in mice are favorable, as is Ames evaluation of mutagenicity. SAR was applied to minimize hERG binding by the RCQ structures; an electrocardiogram study in guinea pigs to test for cardiac response shows a comparable response to that of CQ to high intravenous doses. Rat pharmacokinetics demonstrate good and tunable plasma levels and clearance times. A candidate RCQ drug has been selected and is moving through early preclinical studies.

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ENANTIOMERIC RESOLUTION OF 8-AMINOQUINOLINE ANTIMALARIALS

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Primaquine (PQ), an 8-aminoquinoline (8AQ) antimalarial agent, is the most prescribed drug for the treatment of relapsing malaria and is also an effective prophylactic agent against all plasmodia species. The major drawbacks of this drug are its short half-life and reversible methemoglobinemia and hemolysis in glucose-6-phosphate dehydrogenase deficient subjects. Studies during PQ development showed that the 4-amino-1-methylbutyl side chain on the 8-amino is prerequisite for optimum antimalarial activity. However, this side chain contains an asymmetric center, and conventional methods of preparation yield a racemic mixture. Tafenoquine, currently in clinical development, contains the same side chain and is also being developed as a racemate. Previous studies from our laboratory have shown that enantiomeric resolution of PQ into its individual enantiomers yields two analogs with markedly different efficacy, toxicity, and metabolism profiles. Enantioselective influences on efficacy and toxicity have also been observed with several other 8-AQ analogs with 5-aryloxy or 5-alkoxy substituents and the same 8-amino side chain. We have recently developed evidence that one enantiomer of NPC1161 (an analog with the same side chain) shows a 20-fold increase in efficacy over the other in a mouse causal prophylaxis model, but does not show a commensurate increase in hemolytic potential. In spite of the importance of this issue, it has been difficult to study individual enantiomers or to contemplate their economical development because of the lack of an efficient method to resolve them. A simple and generally applicable procedure has been applied to resolve different classes of 8-aminoquinolines as their phthalimides by fractional crystallization as diastereomeric salts with commercially available chiral organic acids. This procedure can be applied to resolve milligram to kilogram quantities, and affords a viable and economical option for development of new 8-AQ analogs.

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IMIDO-SUBSTITUTED NAPHTHOQUINONES: A NEW CLASS OF POTENTIAL ANTIMALARIALS

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The most dangerous form of human malaria is caused by *Plasmodium falciparum*, accounting for 80% of infections and 90% of deaths. Persistence of this disease in poorer countries of sub-Saharan Africa, Central and South America, and Asia represents a global crisis. Widespread resistance of *P. falciparum* to chloroquine and other commonly available antimalarial drugs exacerbates malaria mortality and intensifies the search for new drugs. Atovaquone, a hydroxynapthoquinone, is effective against multidrug-resistant parasites without *in vitro* evidence of cross-resistance. However, atovaquone is unsuitable for use as a single agent because of the relatively quick emergence of resistance. Several 1,4-napthoquinone derivatives originally investigated as antitumor drugs have been found to interact with novel targets, suggesting that this class of drugs may be effective against drug-resistant *P. falciparum*. For this study, imido-substituted chloro-1,4-naphthoquinone (IMDNQ) analogs have been synthesized and evaluated for antimalarial activity. Our hypothesis is that IMDNQ compounds will affect metabolic pathways distinct from those targeted by existing antimalarials and thus will be less susceptible to existing resistance mechanisms. IMDNQs were screened using a high-throughput malaria SYBR Green I assay. Of eight IMDNQs screened, four had IC50 values <10 ug/ml. Open chain IMDNQ analogs had higher antimalarial activity than cyclic IMDNQ analogs. Additional IMDNQ compounds, particularly open chain analogs, will be screened and the mechanism of action of lead compounds evaluated using a metabolomics approach. Once affected metabolic pathways are defined, evaluation of their direct target(s) and target:drug interactions will be used to further refine the structure of inhibitory compounds. Lead compounds will be evaluated against both drug-sensitive and -resistant parasites to evaluate their potential effectiveness against drug-resistant parasites.

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PROBING THE ANTIMALARIAL MECHANISM OF ACTION OF 1,2,4-TRIOXOLANES IN *PLASMODIUM FALCIPARUM*

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Artemisinin-based endoperoxides are highly potent, structurally complex trioxane antimalarials. Although ferrous activation of the endoperoxide bridge is considered key to drug activity, the mechanism of cytotoxicity remains elusive. Evidence supports a pathway whereby following iron

activation, endoperoxides form damaging free radical metabolites that target parasite macromolecules. Using fluorescent artemisinin analogs, we demonstrated endoperoxide-dependant labeling of neutral lipid bodies associated with the digestive vacuole. We proposed that localization of artemisinin metabolites was due to formation of covalent adducts that further initiated oxidative damage to parasite membranes, as measured by a free radical-sensitive BODIPY probe. A recently developed class of synthetic endoperoxides, comprising a 1,2,4-trioxolane flanked by cyclohexane and adamantane rings, show promise as potent and safe antimalarials. Here, we describe our efforts to similarly probe the localization and reactivity of the trioxolanes in *Plasmodium falciparum*. We applied trioxolane probes tagged with either an adamantane or cyclohexane dansyl group to living malaria parasites for observation by fluorescent microscopy. Our results show that iron activation results in molecular cleavage of the trioxolane producing an alkylating adamantane radical and a cytoplasmic cyclohexanone product. Labeling of neutral lipid bodies by the adamantyl portion of the trioxolanes was similar to that seen with the artemisinin analogs. Our collective findings using fluorescent trioxolanes suggests that endoperoxide-based compounds share a similar mechanism of action in malaria parasites that may involve targeting of neutral lipid bodies.

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A NOVEL CLADE OF EUKARYOTIC RIBONUCLEOTIDE REDUCTASE R2 SUBUNITS IS EXCLUSIVE TO APICOMPLEXAN PARASITES

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Apicomplexans are protist parasites of momentous public health and economic importance. The diseases they cause include malaria, cryptosporidiosis, East Coast fever, babesiosis and toxoplasmosis, and result in millions of deaths and billions of dollars in productivity and property losses each year. Research into new drug targets against these pathogens remains a high priority. Apicomplexan-related diseases may be controlled via inhibition of essential enzymes, provided that these proteins differ significantly in sequence or structure from homologs in their respective hosts. Ribonucleotide reductase (RNR) is one of 57 enzymes prioritized as potential drug targets against *Plasmodium*. RNR provides the only *de novo* means of synthesizing deoxyribonucleotides (dNDPs and dNTPs), the essential precursors for DNA replication and repair. While RNR has long been the target of antibacterial and antiviral therapeutics, targeting this ubiquitous protein to control eukaryotic pathogens may raise toxicity concerns due to its similarity to vertebrate RNR enzymes. The eukaryotic RNR holoenzyme is of the form $\alpha_n\beta_2$, and consists minimally of two large R1 and two small R2 subunits ($\alpha_2\beta_2$). We identified a novel clade of R2 subunits, R2_e2, which forms a sister group to the clade containing all eukaryote standard R2 subunits, R2_e1. Evidence suggests that R2_e2 subunits are functional and yet the amino acid sequence similarity between the two types of R2 subunits is <50%. Remarkably, while most eukaryotic genomes encode two standard R2_e1 proteins, apicomplexans encode one R2_e1 and one R2_e2. In fact, R2_e2 subunits have so far only been identified in apicomplexan genomes. Our results suggest that the novel R2 subunit unique to apicomplexans is a promising candidate for chemotherapeutic-induced inhibition, as it differs greatly from all known vertebrate RNRs and hence can potentially be specifically targeted.

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INTERPLAY BETWEEN COPY NUMBER VARIATION AND ANTIFOLATE RESISTANCE IN *PLASMODIUM FALCIPARUM*

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GTP-cyclohydrolase (*gch1*) is the first and rate-limiting enzyme in the folate biosynthesis pathway and has been found to exhibit extensive copy number variation in isolates from around the globe in areas with a history of longstanding use of antifolates. Specifically in South East Asia, increased *gch1* copy number is associated with increased likelihood of point mutations in dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*), genes which confer resistance to pyrimethamine and sulfadoxine respectively. One hypothesis for this finding is that an increased *gch1* is an adaptive response to compensate for less fit, drug resistant enzymes downstream in the folate pathway. To investigate the effect that *gch1* copy number has on antifolate-resistant parasites, we used a plasmid-based overexpression system, in which we can manipulate *gch1* copy number and expression levels in cultured parasites. We have implemented this system in multiple genetic backgrounds with different drug resistant profiles. We further tested whether the drug sensitivities of our parasite lines were altered using ^{3}H-hypoxanthine drug assays. Our results demonstrate that increases in *gch1* copy number and expression alter drug resistance phenotypes only in parasites bearing a mutant *dhfr*. This suggests that *gch1* amplification increases *dhfr* substrate concentrations relative to that of the inhibitor, thereby relieving the parasite of pyrimethamine pressure and rendering our current antifolate treatments inadequate. In addition, we have found that there is not a linear relationship between *gch1* copy number and expression levels in both isolates from around the globe and in our manipulated parasite lines which warrants further exploration. A greater understanding of the folate pathway and all the factors that play into the development of drug resistance is key to development of new drugs targeting this pathway and to understanding in general how the parasite can adjust to different drug pressures through both point mutations and copy number variation.

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WANING EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP) WITH SULPHADOXINE-PYRIMETHAMINE (SP) IN THE PRESENCE OF HIGH SP RESISTANCE IN MALAWI

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Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended by the World Health Organization for the control of malaria in pregnancy in sub-Saharan Africa. Malawi was the first country to introduce IPTp with SP in 1993. Parasite resistance has compromised the efficacy of SP in the case-management of symptomatic children, but SP remained effective for IPTp in many areas of Africa. We conducted an observational study of women delivering in an area with high SP resistance (frequency of quintuple dhps/dhfr mutant haplotype >95%) in Blantyre district, Malawi to study the effect of SP resistance on

the efficacy of IPT-SP in preventing placental malaria and preterm delivery or low birth weight. Previous in-vivo studies in this area indicated that 50% of asymptomatic parasitaemic HIV-negative primi+secundigravidae (G1+2) who received IPT-SP were parasitaemic again within 42 days. Between Dec 2009 and Sep 2010, 780 HIV-negative women delivered (418 G1+2 and 362 multi-gravidae [G3+]), of whom 2.4%, 12.7%, 51.2% and 33.7% had received none, 1, 2, or 3 or more doses of IPTp-SP and 66.6% reported using a bednet. Among G1+2, the prevalence of placental malaria detected by histopathology or RDT was similar in each dose group (44%; 36%; 41%; 50% in the 0, 1, 2, 3 dose group respectively). Among G3+ the prevalence was lower among women receiving IPT, but there was no difference with each incremental dose (30%; 13%; 13%; 11%). The frequency of preterm delivery or LBW was similar in all dose groups among G1+2. Molecular analyses for SP resistance-associated mutations in dhps 436, 437, 540 and 581, dhfr 51, 59 and 164 and pfmrp1 1466 are ongoing and will be presented. These preliminary results suggest an absence of a beneficial impact of IPTp-SP among G1+2 protected by ITNs in this area with high grade SP resistance and near saturation of the quintuple dhps/dhfr haplotype. This raises concern about the longevity of IPTp-SP in southern Malawi and stresses the need to explore alternative drugs or strategies to replace SP or IPTp.

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MONITORING OF DRUG RESISTANCE AFTER INTERMITTENT PREVENTIVE TREATMENT FOR INFANTS AND CHILDREN (IPTI/C) IN SENEGAL

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In 2006, the health authorities of Senegal changed drug policy from sulfadoxine-pyrimethamine (SP)/amodiaquine (AQ) to the Artemisinin Combination Therapy (ACT) AQ/artesunate as first-line treatment against uncomplicated *falciparum* malaria. This was done due to reports of *Plasmodium falciparum* widespread resistance to SP and AQ. Currently, SP is still used for intermittent preventive treatment (IPT) as a method for reducing malaria morbidity and mortality and is being used in pregnant women (IPTp), infants (IPTi) and is being studied for children (IPTc). This study was undertaken to examine the impact SP use for IPTi and IPTc on the frequency of SP-resistant related haplotypes in the *Plasmodium falciparum* genes, Pfdhfr and Pfdhps. Samples were collected during a cross sectional survey in 2010 involving children under five years old living in three health districts located in the Southern Senegal where malaria transmission is high. Overall, 257 samples were *P. falciparum* positive. Among them, 176 individuals had received SP two years ago through IPTi in two of the districts while 81 did not. All positive samples were analyzed to determine the frequency of SP-resistance related haplotypes in Pfdhfr and Pfdhps based on results obtained by nested PCR followed by sequence-specific oligonucleotide probe (SSOP)-ELISA. The triple mutant Pfdhfr CIRNI haplotype dominated in both groups [IPTi+ (58%) and IPTi- (50%)]. The double mutant Pfdhfr CNRNI haplotype was also found with a frequency less than 5% in both groups. For Pfdhps, the wild type haplotype SAKAA dominated the control group with 28% (23/81) against 15% (26/176) with a significant difference (p=0.036). The double mutant Pfdhps haplotypes SGEAA and AGKAS were found in our study with a frequency less than 5% in both groups. The single mutant SGKAA haplotype was more frequent in IPTi+ group (30%) than in IPT- group (5%) the difference is not significant (p=6x10-6). In conclusion, the present study indicates that using SP for IPTi does not select resistant parasites when follow up is performed long term. Base on WHO recommendation, SP can still be use as IPTi in Senegal because of the very low frequency of Pfdhps haplotype SGEAA (<5%)

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THE RETURN OF WIDESPREAD CHLOROQUINE SENSITIVE *PLASMODIUM FALCIPARUM* TO MALAWI

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Since chloroquine-resistant *falciparum* malaria became pervasive in Africa, the reemergence of predominantly chloroquine-sensitive parasite populations has been documented in Blantyre, Malawi, an urban center in Eastern Africa. This resurgence of sensitive parasites followed a change in national treatment policy from chloroquine to sulfadoxine-pyrimethamine in 1993, and treatment efficacy of 99% was demonstrated in 2005. Studies in other areas of Malawi report varying results on resistance levels outside of this population center. This study evaluated the prevalence of chloroquine drug resistance using a marker in the *Plasmodium falciparum* chloroquine resistance transporter *(pfcrt)* gene throughout the country, including rural areas and districts bordering countries where chloroquine use persisted much later than 1993. Dried blood spots were collected from children aged five years or less using two-stage cluster sampling in eight districts across Malawi in 2009. Samples with *P. falciparum* parasitemia on microscopy underwent PCR amplification and pyrosequencing of the *pfcrt* gene 76 amino acid region to determine chloroquine resistance status. Of 7145 samples collected, 1168 were found to have parasitemia by light microscopy. Of 696 with sufficient DNA for sequencing only 2 were found to have the chloroquine resistance genotype. This translates to an overall proportion of infections with detectable resistance of 0.003 (95% CI:-0.001,0.007) and a proportion of 0.167 (95% CI: -0.262,0.595) in Karonga and 0.007 (95% CI: -0.007, 0.020) in Mwanza, the two districts where resistant samples were found. Sampling over a wide geographic region of Malawi, including higher risk sites for ongoing resistance such as border areas indicates that chloroquine-susceptible malaria now predominates the parasite population in this country. A very small subpopulation of resistant parasite nevertheless appears to persist within this population, suggesting that resumption of chloroquine use might be quickly followed by selection and increasing prevalence of chloroquine-resistant parasites.

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A NETWORK-BASED APPROACH TO PROBING THE METABOLIC PATHWAYS OF *PLASMODIUM FALCIPARUM*

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There is a growing demand for high resolution data to quantify and characterize the enzymic and metabolitic status of the human malaria parasite, *Plasmodium falciparum*. The resolution of a metabolic network offers insights into the life cycle and pathophysiology of the parasite. Since metabolites are the ultimate cellular readout, investigating the

global metabolic flux regulation of an organism is generally more informative than measuring mRNA levels. Our approach is enhanced by the incorporation of network theory and graphs that model the interconnected and sequential conversions of compounds in metabolic pathways. The profile of individual metabolite levels inherited in progeny of a genetic cross can serve as a phenotype to uncover genetic factors underpinning parasite physiology using quantitative trait loci (QTL) mapping. We extracted metabolites from the parents and progeny of HB3 × Dd2 and 7G8 × GB4 genetic crosses of *P. falciparum* at three erythrocytic cell cycle stages and constructed a metabolic network using Pearson's correlation of metabolite levels obtained from LC-MS. Individual mass signatures, the vast majority still unidentified, map to all chromosomes in the genome in an asymmetrical manner such that a few loci influence the levels of many compounds while other loci affect none. Our network approach does not rely on QTL; however, the network modularity of clustering patterns of compounds can be used to evaluate the significance of QTL. We investigate whether co-mapping compounds from QTL hotspot regions also cluster together in the network. Finally, the network provides an interpretive framework for the prioritization of these unknown compounds by clustering metabolites involved in specific pathways and by leveraging information about the known metabolites. These studies establish a framework to construct and analyze the metabolite network in *P. falciparum*, and will ultimately provide useful insights about antimalarial drug resistance and prospective targets.

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GAMETOCYTE CLEARANCE DYNAMICS FOLLOWING ORAL ARTESUNATE TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN MALI, WEST AFRICA

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Artemisinin-based combination therapies (ACTs) reduce *Plasmodium falciparum* gametocyte carriage, but their true effect on gametocytes and on transmission potential is not fully understood. A better understanding of gametocyte dynamics *in vivo* in the presence of artemisininsis needed. One hundred children aged 1-10 years presenting with uncomplicated *falciparum* malaria to a sentinel site clinic in Bougoula-Hameau, Mali were treated with seven days of directly-observed oral artesunate therapy from December 2010 to February, 2011. Thick and thin blood smears were prepared and read every 8 hours until three consecutive slides were negative for asexual *falciparum* parasites. Gametocytes were quantified by two trained microscopists using standard WHO procedures. Gametocyte carriage and density were compared at 0, 1, 2, 3, 7, 14, 21 and 28 days after treatment initiation using the chi-square test and the student's t-test, respectively. Of 92 children in the final analysis, 21 (22.83%) were gametocyte carriers at the time of treatment initiation. The proportion of gametocyte carriers was unchanged at the end of treatment (day 7, 23.91%, p=1.0) and did not significantly decline until day 21 of follow-up (6.52%, p=0.003). The mean gametocyte density at inclusion, 11.78 gametocytes/µl, also remained unchanged at the end of treatment (13.25 gametocytes/µl, $p > 0.05$) and only dropped significantly at day 28 of follow-up (0.62 gametocytes/µl, p=0.01). Among carriers at inclusion, the median clearance time was 14 days. Among non-carriers at inclusion, 6 (8.11%) became carriers by day 7. Artesunate decreased gametocyte carriage and gametocyte density by the end of the 28-day follow up. However, artesunate did not prevent the maturation of young gametocytes to circulating stage V, as evaluated by standard microscopy. More sensitive gametocyte detection methods may better characterize these dynamics. Further work is needed to determine the role sequestered gametocytes may play in the persistence of peripheral gametocytemia after artemisinin-based treatment initiation.

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RESEARCH CAPACITY DEVELOPMENT FROM SCRATCH: THE EXPERIENCE OF THE WEST AFRICAN NETWORK FOR CLINICAL STUDIES OF ANTIMALARIAL DRUGS (WANECAM)

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Malaria remains a major public health problem in much of sub-Saharan Africa. Yet there is little data on the epidemiology, transmission and drug resistance in many areas of the Continent. To address these issues in Guinea, West Africa, we are building human capacity, infrastructure and the regulatory frame work necessary for conducting state of the art clinical research. A TDR initiative of 1997 selected a young Guinean Scientists with strong potential for research. The scientist received nearly 8 years of training in the laboratory and in the field in France and in Mali leading to a MSc and a PhD degrees in Parasitology. He was then invited to join the EDCTP funded WANECAM project. A site assessment visit by senior members of the Network helped in streamlining the needs in human capacity, infrastructure and regulatory environment. A team of 8 young scientists with little or no experience in research was recruited. The team received intensive short-term training in GCP, ethics, computer skills and clinical studies. Training included short-term workshops both in Guinea and abroad, the posting of experienced Malian scientists in Guinea for extended periods and short visits by experienced senior staff from the other network members. Two students were enrolled for MSc training in Burkina Faso and in Mali. Two 4-weel drive vehicles were purchased. A vacant building was obtained from the Government of Guinea and refurbished into a brand new polyvalent laboratory. As a result, the first malaria entomology survey was conducted. A prospective longitudinal study on references ranges of biological parameters, age specific incidence and drug resistance is underway. A solid and emerging malaria research team is now in the building in Guinea. This experience underlines that capacity development in developing countries is a long-term investment on the scientists, the environment, and the physical infrastructure.

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EFFICACY OF ARTEMETHER-LUMEFANTRINE AND ARTESUNATE-AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* INFECTION IN TANZANIA

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Following the development of drug resistance to anti-malarial first line treatment of uncomplicated malaria with sulfadoxine-pyrimethamine (SP) by *Plasmodium falciparum* in mainland Tanzania the ministry of health and social welfare (MOHSW) introduced artemisinin combination therapy (ACT) with artemether-lumefantrine (ALu) as first line treatment for the treatment of uncomplicated *falciparum* malaria in 2006. There

is growing evidence suggesting that malaria cases over the past three years and entomological inoculation rates (EIR) that are currently monitored in most parts of Tanzania are declining. Despite good malaria control achievements, there is a threat of ACT drug resistance. Due to recent report on the emerging drug resistance to ACT along the Thai-Cambodia border it is critical to our region to monitor the spread of drug resistance to ACT. We set up to conduct an invivo monitoring study at four country-wide representative National Malaria Control Programme (NMCP)'s sentinel sites in May-August 2011 to assess efficacy of ALu and amodiaquine-artesunate both anti-malaria first line in Mainland Tanzania and Zanzibar respectively. The study sites are Mlimba, Mkuzi, Kibaha, and Muheza in the mainland Tanzania. Participants are febrile patients aged 6-59 months presenting at the health facility to be followed up during 28 days to elicit treatment performance. Results of this study will be out by the time of American Society of Tropical Medicine and Hygiene conference in November 2011. We will elucidate the occurrence of drug resistance by PCR using msp1 and glurp. As some of the current molecular genotyping malaria tools are based on SP which is also used for chemoprophylaxis (IPTp or IPTi) we will also generate data on molecular markers (dhfr and dhps) for SP resistance. This analysis will assist to monitor the evolution, spread and intensification of ACT and SP resistance. Results from this study will be used to assist the MOHSW to assess the current national treatment guidelines for uncomplicated. *Falciparum* malaria.

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EFFICACY OF FIXED-DOSE COMBINATION ARTESUNATE-AMODIAQUINE VERSUS ARTHEMETHER-LUMEFANTRINE FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN UNDER FIVE: A RANDOMIZED NON INFERIORITY TRIAL IN DEMOCRATIC REPUBLIC OF CONGO

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Until now, only a limited number of studies have been published in Central Africa measuring the efficacy of artemisinin combination therapies (ACTs) since their introduction. The Democratic Republic of Congo (DRC), one of the largest countries in the region, adopted artesunate and amodiaquine (ASAQ) as first line antimalarial treatment in 2005. We conducted a randomised open-label non-inferiority trial, enrolling children aged 6-59 months with uncomplicated *P. falciparum* malaria in Pweto district, Katanga province. Patients were randomly allocated into one of the two regimens, fixed-dose formulation ASAQ or artemether-lumefantrine (AL). We analyzed the risk of recurrent parasitemia by day 42 adjusted by PCR genotyping, expressed as estimates of failure from survival analysis and as simple proportions (per protocol). Of 1993 children who were referred to the study clinic between April 2008 and March 2009, we enrolled 301 children: 156 with ASAQ and 145 with AL. The proportion of patients with parasitemia were low in both groups at D2 and D3: 6.0% (9/150) in the ASAQ arm and 4.9% (7/143) in the AL arm; and 0.6% (1/150) and 0.7% (1/143) respectively. After PCR correction, cure rates were 98.3% (95%CI, 94.1-99.8) in the ASAQ group and 99.1% (95%CI, 94.9-99.9) in the AL group (difference -0.7%, one sided 95%CI -3.1). Kaplan-Meier PCR-adjusted cure rates were similar: ASAQ, 98.4% (95%CI, 93.8-99.6) vs AL, 99.2% (95%CI, 94.3-99.9). Both treatment regimens were well tolerated. The results show that ASAQ was not inferior to AL and that both ACTs were highly effective as first-line malaria treatment in this area. The logistical constraints of a remote site and the slow recruitment of comfirmed cases were among the main challenges and increased substantially the cost of the study. The recommended therapeutic efficacy surveys throughout the territory at repeated intervals are difficult to achieve considering the logistical challenges and the limited technical capacity in a country like DRC.

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MONITORING THE EFFICACY AND SAFETY OF ACTS TO TREAT UNCOMPLICATED MALARIA IN BOBO-DIOULASSO, BURKINA FASO

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Malaria in Burkina remains the major public health compromising therefore the development of the country. Since 2005, the national malaria control program advocated artéméther-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) respectively as first and second lines for the treatment of uncomplicated *falciparum* malaria. Monitoring efficacies of these artemisinin based combination therapies play a major role in early detection and containment of resistance. We compared efficacies of AL and ASAQ for the treatment of uncomplicated *falciparum* malaria in two randomized trials with patients aged over 6 months. Outcome of treatment were defined according to standard WHO classification, ETF, LCF, LPF and ACPR. Genotyping to distinguish recrudescence from new infections is ongoing. Overall, 618 patients included in both studies completed their follow-up. We did not noted any ETF and at day 28, risk of recurrent infection were 9/66 (13.6%) in AL group compared to 4/62 (6.5%) in ASAQ group in 2009 and 46/211 (21.8%) compared to 20/215 (9.3%) in 2010. Most of treatment failures were new infections and PCR corrected ACPR were similar for both drugs in the two studies. No serious adverse event related to the studies drugs was recorded. Known polymorphisms-mediating resistance in pfcrt and pfmdr1 were not associated with treatment failure. All study drugs have shown excellent efficacy and safety in treating uncomplicated *falciparum* malaria in Burkina but the concern might be the reported resistance-mediating polymorphisms selection by the partner drugs following treatment.

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ESTIMATING SELECTION ON *PLASMODIUM FALCIPARUM* DRUG RESISTANCE ALLELES IN AN ENDEMIC POPULATION OVER A 25-YEAR PERIOD

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Using archived blood samples, we surveyed the changes in drug resistance alleles in The Gambia over a 25 year period from the time when resistance was unknown locally (in 1984) through periods of gradual failure of chloroquine therapy and increasing use of sulphadoxine-pyrimethamine until eventual introduction of artemisinin combination therapy (in 2008). At the first survey there were no drug resistance alleles detected at two of the loci (*crt* and *dhps*) and very few isolates contained resistance alleles at the other two loci (*mdr1* and *dhfr*). Proportions of isolates with resistance alleles increased progressively over subsequent surveys, reaching peaks for the chloroquine resistance alleles *crt* 76T (76%) and *mdr1* 86N (78%) in the year 2000, and for antifolate resistance *dhfr* alleles (94%, mostly as a triple combination of 51I, 59C and 108N) and *dhps* 437G (86%) in 2007 and 2008 respectively (the *dhps* resistance allele 540E was not present in any of 623 isolates genotyped over the whole period). To estimate changes in allele frequencies over time, we counted one allele at each locus per isolate, randomly sampling when there were mixed genotypes, and estimated 95% confidence intervals based on sample sizes in each year. Changes in allele frequencies occurred at different times and rates over the period of survey, and the data fit closely a very simple model for each locus with assumed fitness costs and a change in selection

coefficients reflecting historical change in therapeutic use. We explore the fit between these historical selection data and signatures of selection at these loci that can be derived from genome wide polymorphism data in a population sample taken at the end of the period.

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ALIGNMENT AND GENE SET ENRICHMENT ANALYSIS OF TIME-COURSE PROFILES OF RECOMBINANT PFMDR1 AND PFCRT-MODIFIED *PLASMODIUM FALCIPARUM* PARASITE LINES

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Transcriptional profiling studies of the intraerythrocytic developmental cycle (IDC) of *Plasmodium falciparum* have revealed a unique transcriptome characterized by a continuous cascade of expression. Here we present a quantitative time-course analysis of the gene expression levels of 6 strains that differ in 2 key antimalarial resistance determinants, pfmdr1 and pfcrt. Continuous expression profiles were imputed from the 8 time points sampled for each strain and then aligned through dynamic time warping. Transcriptional differences were elaborated at both the gene and gene set level using a novel algorithm that measures gene set enrichment at many discrete time points along the imputed and aligned expression profiles. Significantly up or down-regulated gene sets were identified in each comparison along with the time period of maximal enrichment. We present software to visualize the complete aligned expression profiles of each strain in 2 or 3 dimensions, facilitating comparison of individual time points as well as the full time-series. Comparison of our alignment methods with conventional techniques underscores the vital role that temporal alignment plays in discriminating genuine biological signal from the transcriptional noise created by gene expression cascades peaking at different time points and durations. Together, our data and software tools provide a window into the rich transcriptional complexity of *P. falciparum* parasites by allowing the alignment and comparison of strains that differ in fitness and therefore progress through the IDC at varying rates.

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THE INTERACTION BETWEEN MALARIA PARASITES AND BLOOD GROUPS IN PORT HARCOURT, NIGERIA

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The impact of malaria on the public health of resource-limited economies of the world is still a major problem particularly in Africa where 89% of all malarial deaths occur. The pathogenesis of *Plasmodium* infection entails merozoite invasion of erythrocyte, which implies an implicit interaction between the red cell membrane proteins and the invading plasmodium antigens. Blood group antigens serve as genetic markers of several clinical conditions including malaria; the clearest example being the well elucidated inter-relationship between *Plasmodium vivax* and the Duffy antigen. Thus, the products of research on blood groups and malaria may have a potential impact on the development of new anti-malarial chemotherapy, vaccines and reduction of the global burden of malaria. This study was designed to investigate the link between blood groups and different malaria parasites in Port Harcourt, Nigeria which is the centre of the oil and gas industry in West Africa. Furthermore, we will investigate the incidence of *Plasmodium ovale* and the specificity of the parasite strain in relation to various blood groups in this environment. Thick blood smears and filter paper blood spots were made from finger-prick for microscopy and molecular genotyping of parasite strains. Two hundred and forty six participants: 142 males (57.72%) and 104 females (42.28%) aged 16-60 years attending the Braithwaite Memorial Hospital and blood donors presenting at the University of Port Harcourt Teaching Hospital Blood Bank were enrolled into the study. Preliminary results showed that 207 (84.1%) were positive for *Plasmodium falciparum* while 39 (15.9%) were negative by microscopy. However prevalence of other species is expected from the PCR genotyping. Results of the blood group screening showed that blood group O Rh positive was the highest with 163 (66.2%) followed by blood group A Rh positive 43 (17.5%), B Rh positive 26 (10.6%), O Rh negative 7 (2.85%), AB Rh positive 5 (2.03%), B Rh negative 1 (0.41%) and A Rh negative 1(0.41%).

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BASOPHIL REACTIVITY IS ASSOCIATED WITH MALARIA SEVERITY AND PFTCTP

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Understanding of malaria immune-pathogenesis will lead to the identification of new therapeutic strategies aimed at improving recovery. Recent findings have suggested common mechanisms in malaria pathogenesis and allergy. Elevation of IgE levels has been associated with malaria infection, but their role remains unclear. Similarly, a parasite-derived histamine releasing factor (PfTCTP) was found at high level in serum from patients but *in vivo* effects are unknown. To address these questions, we conducted a clinical study in Dakar (Senegal). *Plasmodium falciparum* infected patients with mild (MM, n=19) or severe (SM, n=9) symptoms were enrolled and compared with healthy controls (HC, n=38). We performed basophil activation tests on whole blood samples based on CD203c expression to analyse allergic response. Basophils from MM patients showed significant lower baseline levels of CD203c expression, compared to SM and HC. Basophils from SM patients were characterized by a higher reactivity to A23187, haemozoin and anti-IgE stimulation. Ex vivo priming of basophils with recombinant human or PfTCTP before stimulation with anti-IgE induced either an enhancement or an unexpected decrease in activation (mostly in MM and HC patients). The decrease in basophil activation, previously described as an "overstimulation", suggests a better ability of HC and MM patients to control allergic response following excessive stimulation. IgE levels were also higher in malaria patients than in healthy ones, but were not related to basophil responses. Indeed the reactivity of basophils from malaria patients was positively related to the presence of circulating PfTCTP or for SM, to the lack of anti-PfTCTP IgG. Altogether these data revealed a high reactivity of basophils during SM which could explain the high level of histamine reported during SM, likely contributing to blood-brain-barrier impairment. These findings support an involvement of allergic immune responses in malaria pathogenesis which can be exacerbated by the proinflammatory environment and PfTCTP.

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IPT/C: PREVALENCE OF ANTIBODY AMA-1 AND MSP-1(19) IN THREE AREA IN SENEGAL

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Malaria remains a major disease in many African countries. Nowadays, many strategies such as IPTic /SP are used for prevention in children. But SP- resistant parasites can compromise this strategy. To evaluate the impact of IPTic /SP on antigenic variation in rural areas of three districts, all children aged 5mths-10years, in Senegal. In 2009, to assess the role of

serological markers in evaluating malaria transmission, filter papers were collected from children under 10 years. Filter blood spot papers were collected from 5833 people from Mbour, Bambey and Fatick to assess the prevalence of antibodies to two *Plasmodium falciparum* antigens MSP-1(19) and AMA-1. Seropositivity to *P. falciparum* MSP-1(19) was 15.5 % and 26.7% to AMA-1. MSP-1(19) is lower than AMA-1. Fatick presents most of positive children who answer to antibody. Also in Fatick the young children have least antibody. Seroepidemiology can provide key information on malaria transmission for control programmes, when parasite rates are low.

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COMPARATIVE PROTEOMIC ASSESSMENT OF *PLASMODIUM CHABAUDI ADAMI* AS-INFECTED AND NAÏVE MOUSE SERUM TO IDENTIFY CANDIDATE CFF PROTEINS

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The identity of serum crisis form factor (CFF) has remained elusive for decades after its initial characterization as a factor in human immune sera able to inhibit growth and cause the intraerythrocytic degradation of the malaria parasite, *Plasmodium falciparum*, in culture. CFF is named for the association of its coincident presence with the immunological crisis leading to resolution of infection and is inducible through artificial immune stimulation in rabbit and rodent models by treatments such as BCG/LPS and malaria infection. CFF has been described in the serum of some individuals with apparent resistance to malaria symptoms and may provide a novel mechanism of natural immunity. Identification of CFF would enhance our understanding of how immune system components interact with a *P. falciparum* infection to produce acquired immunity, which is a critical component of the current investigation into a malaria vaccine. In this study, we used the C57BL/6 mouse model inoculated with *P. chabaudi adami* AS to induce serum CFF, as documented by inhibition of *P. falciparum* growth in culture and the appearance of classic CFF responses in microscopic findings. We isolated the low abundance protein fraction of these CFF mouse sera using IgY depletion. Proteomic analysis using MALDI and LC-QToF was conducted on depleted serum from the C57BL/6 *P. chabaudi adami* AS model and non-inhibitory serum from naïve mice. Protein differences were quantified to discover proteins that were present in the CFF serum and absent from naïve serum. This analysis highlighted 68 proteins as either up-regulated or unique to CFF serum, and a qualitative analysis revealed potential CFF candidates. This study provides new insight into the etiology of CFF and host serum protein changes during a malaria infection.

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PEDIATRIC MALARIAL ANEMIA SEVERITY IS DEFINED BY ELEVATED LEVELS OF CIRCULATING MEMORY CD4 T CELLS PRODUCING IL-17

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In *Plasmodium falciparum* holoendemic transmission regions of western Kenya, severe pediatric malaria manifests as severe malarial anemia (SMA). We hypothesized that children presenting with SMA would have chronic immune responses characterized by effector/central memory CD4+ T cells producing interferon (IFN)-γ and/or interleukin (IL)-17 that suppressed their erythropoietic responses. We therefore characterized the CD4+ T cell populations and their intracellular IFN-γ and IL-17 production in healthy controls [HC; hemoglobin (Hb)≥11.0g/dL, without parasitemia, n=13] and febrile children with differing levels of malarial anemia severities and any density parasitemia: uncomplicated malaria (UM, Hb≥11.0g/dL, n=140; mild malarial anemia (M/MA, Hb≥8.0≤10.9g/dL, n=23); and SMA (Hb<6.0g/dL, n=23). Across group comparisons revealed that children with SMA had elevated effector memory (T_{EM}) (CD4+CD62L-IFN-γ+) cells [median (IQR) 92.60% (7.50)] relative to the HC [75.00% (19.10)], UM [62.80% (15.50)], and M/MA [66.70% (25.00), *P<0.001*] groups., T_{EM} (CD4+CCR7-IL-17+) cells were also highest in the SMA group [87.15% (5.80)] compared to the HC [44.80% (15.40)], UM [58.05% (13.40)], and M/MA [78.40% (10.20), *P*<0.001] groups. In addition, the SMA group had higher integrated mean fluorescence intensity (iMFI) for IFN-γ in T_{EM} cells [HC, 1628.48 (719.60); UM, 1521.31 (852.20); M/MA, 1994.33 (1397.50); and SMA, 3429.30 (1758.20) (*P*<0.001)]. The iMFI of IL-17 in T_{EM} cells increased with disease progression towards SMA [HC, 1386.00 (1293.70); UM, 1895.00 (634.70); M/MA, 2716.55 (2567.30); and SMA, 5718.80 (1540.1), *P*<0.001]. Moreover, the iMFI of IFN-γ and IL-17 in T_{EM} cells were negatively correlated with Hb levels (r=-0.600, *P*<0.001; and r=-0.690, *P*<0.001, respectively). Our findings suggest the involvement of T_{EM} producing IFN-γ and/or IL-17 in pediatric SMA pathogenesis.

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IDENTIFICATION OF HOST TRANSCRIPTIONAL PROFILES ASSOCIATED WITH ASYMPTOMATIC MALARIA AFTER A BOUT OF SEVERE MALARIA

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Clinical signs of *Plasmodium falciparum* can range from cerebral malaria to asymptomatic carriage. Prior exposure and host genetics will alter clinical presentation; however, the mechanisms associated with clinical presentation are not fully characterized. To explore the role of the host response across clinical phenotypes, we studied human whole genome transcription expression profiles from children with cerebral malaria admitted to the Blantyre Malaria Project during a single malaria season. Survivors are invited to return for a one-month follow-up visit, and we analyzed samples from survivors found to have asymptomatic malaria infections at that time. Whole blood (2-3 mL) was collected, stabilized in Tri-Reagent, and frozen at -80°C at the time of admission and at follow up (day 30). RNA was isolated from sixty severe disease samples and five follow-up matched samples. RNA was hybridized to Affymetrix GeneChip Human 1.0 ST Arrays. For the paired analysis of the severe and asymptomatic samples (n=5), we identified significantly differential gene sets using GSEA (GenePattern, Cambridge, MA) software. The severe disease presentation in the matched samples was significantly associated with olfactory sensory transcripts (GO:olfactory sensory receptor activity). The olfactory bulb is unique to the central nervous system in that it has an external component. We speculate that our peripheral blood analysis may be detecting this peripheral component of the brain, reflecting the central nervous system abnormalities involved in cerebral malaria. GO sets significantly upregulated in samples derived from the asymptomatic presentation reflect immune system upregulation (GO:regulation of the immune system processes; GO:regulation of leukocyte differentiation). This is the first report that captures the peripheral blood transcriptomes during a bout of severe malaria and during a subsequent asymptomatic infection

and may provide insight into host response associated with clinical presentation to inform pathogenesis/immunity models and potential targets of intervention.

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CD11C EXPRESSION DEFINES MULTIPOTENT EFFECTOR MEMORY CD8 T CELLS INDUCED BY GENETICALLY-ATTENUATED MALARIA VACCINES

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Vaccination with live, genetically-attenuated *Plasmodium yoelii* parasites (PyGAPs) can induce long-lasting sterile protection against liver stage malaria in mice with just one dose. The underlying mechanisms mediating this protective immune response are not fully understood, but further characterization will be vital for guiding future vaccine design. Previous work from our lab demonstrates that protective immunity following PyGAP immunization is completely dependent on CD8 T cells, partially dependent on IFN-γ and perforin, and likely mediated by direct cytotoxic killing of parasite-infected hepatocytes. In addition, protective efficacy correlates with expansion of effector memory CD8 T cells in the liver. We went on to further characterize vaccine-induced changes in the T cell phenotype and found significant up-regulation of CD11c on CD3+CD8+ T cells in the liver, spleen and peripheral blood. As much as 50% of CD8 T cells co-expressed CD11c in the liver, which is the site of infection, and expansion of the CD11c+ CD8 T cell population correlated with protective efficacy following various vaccine regimens. CD11c expression was specifically induced on T cells from immunized mice but not from control mice following co-culture with malaria-infected hepatocytes. Further analysis demonstrated that these CD11c+ T cells are predominantly CD11a+ $CD44^{hi}$ CD62L-, indicating that they are antigen-experienced, effector memory cells. Following *in vitro* re-stimulation with malaria-infected hepatocytes, CD11c+ CD8 T cells expressed multiple inflammatory cytokines and cytotoxicity markers, including IFNγ, TNFα, IL-2, perforin and CD107a. CD11c- CD8 T cells, on the other hand, expressed negligible amounts of inflammatory cytokines and cytotoxicity markers, indicating that CD11c expression accurately defines multifunctional effector CD8 T cells. Surprisingly, we found that CD11c+ CD8 T cells also express other antigen-presenting cell (APC) markers, including MHC class II, CD80 and CD86, suggesting that these cells may have an unusual APC-like phenotype. Taken together, our data demonstrate that CD11c+ CD8 T cells are multipotent effector memory cells that are likely to mediate the protective immune response against liver stage malaria infection following PyGAP vaccination.

1197

DECREASED SYSTEMIC PROSTAGLANDIN (PG)-E_2 AND CYCLOOXYGENASE (COX)-2 GENE EXPRESSION IN CHILDREN WITH SEVERE MALARIA ANEMIA AND CO-INFECTION WITH HIV-1 OR BACTEREMIA

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In malaria endemic regions of western Kenya, *Plasmodium falciparum* malaria manifests clinically as severe malarial anemia [SMA; hemoglobin (Hb)<6.0g/dL, any density parasitemia]. Although we have previously shown that prostaglandin (PG)-E_2, cyclooxygenase (COX)-2 transcripts and protein levels are reduced in children with severe and cerebral malaria, the impact of HIV-1 and bacteremia co-infections, and *in vivo* malaria pigment containing monocytes (PCM) on systemic PGE_2 production and COX-2 mRNA expression in children with SMA has not been investigated. As such, we investigated plasma and urine PGE_2 (measured as bicyclo-PGE_2) and COX-2 mRNA expression in children with clinical malaria (n=74) and those co-infected with either HIV-1 (*Pf*+/HIV-1+; n=8) or bacteremia (*Pf*+/bacteremia+; n=19). Plasma (P=0.001) and urinary (P<0.001) PGE_2 levels were decreased in children with SMA relative to the non-SMA (Hb≥6.0g/dL, any density parasitemia) group. Additionally, PGE_2 levels were lower in *Pf*+/HIV-1+ children in plasma (P<0.001) and urine (P=0.007), as well as *Pf*+/bacteremia+ children in plasma (P<0.001) and urine (P=0.173), relative to those with malaria infection alone. PGE_2 increased with increasing hemoglobin levels in children with malaria (plasma; r=0.363, P=0.002 and urine; r=0.500, P=0.001), and in co-infected children (*Pf*+/HIV-1+; r=0.819, P=0.013 and *Pf*+/bacteremia+; r=0.595, P=0.007). Additional analyses demonstrated decreasing PGE_2 levels with increasing PCM in plasma (P=0.031) and urine (P=0.070). COX-2 mRNA expression was decreased in children with SMA relative to the non-SMA group (P=0.011) and in *Pf*+/bacteremia+ (P=0.033) and *Pf*+/HIV-1+ children (P=0.118) relative to those with malaria alone. Taken together, results demonstrate that SMA is associated with decreased PGE_2 and COX-2 gene expression, and is further augmented by co-infections (HIV-1 and bacteremia), driven in part, by naturally acquired malarial pigment by monocytes.

1198

LIVER-RESIDENT CD8+ T CELLS INDUCED BY RADIATION-ATTENUATED *PLASMODIUM* SPOROZOITES

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Memory CD8+ T cells induced by malaria sporozoites home to the liver and eliminate parasite-infected hepatocytes. While memory T cells residing in lymph nodes, spleen, lung and peripheral blood are polyfunctional, capable of mediating cytotoxicity and producing multiple cytokines, the liver-resident memory cells exhibit a unique monofunctional profile with normal cytotoxic activity but minimal cytokine production. This phenotype is specifically induced by parasites but not viruses expressing the same epitope. The liver-resident memory cells are not exhausted, anergic or senescent, albeit their proliferation after *in vivo* antigen re-exposure is markedly reduced. Importantly, these cells undergo vigorous homeostatic proliferation, display normal *in vivo* cytotoxic activity and inhibit parasite development in hepatocytes. Surprisingly, these cells are fully capable of producing IFN-γ transcripts but translation occurs only in response to TCR-independent stimuli. These results suggest that parasite-induced liver-resident memory CD8+ T-cells represent a distinct terminal effector lineage characterized by a monofunctional profile maintained in part through translational control of cytokine production.

1199

USING PROTEIN ARRAYS FOR ANTIBODY PROFILING AND DISEASE STRATIFICATION IN MALARIA INFECTION

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The prevalence of mixed-species malaria infections was underestimated until more sensitive detection methods, such as PCR-based diagnosis, were introduced for epidemiological studies in malaria endemic areas. In the era of malaria elimination, improved diagnostic tools are required to enable targeted treatment of infected individuals as well as effective mass screening for the detection of very low parasite densities to monitor

transmission reduction. Serological markers represent a promising tool for diagnostics and surveillance, especially for *Plasmodium vivax*, for which current rapid diagnostic tests are less effective. A comprehensive characterization of the antibody response to blood stage malaria for both *P. falciparum* and *P. vivax* is required for the discovery of novel markers of both single and mixed clinical infections, as well as asymptomatic low density infections. Using recent developments in malaria genomic sequencing, proteomics, bioinformatics, high throughput cloning and proteome microarray fabrication technologies, we have constructed a blood stage proteome antigen array with a total of 4,000 recombinant proteins, which are the expression products of approximately 2,000 *P. falciparum* and 2,000 *P. vivax* blood stage ORFs. After recombinant cloning proteins were expressed using an E. coli based cell free expression system and printed directly on the nitrocellulose coated microarray slides without purification. Using this protein chip, sera from both symptomatic and asymptomatic children with *P. falciparum* and/or *P. vivax* infections from Papua New Guinea were screened. This approach will provide new insights into the correlation between antibody profiles and disease states that will lead to the characterization of serological correlates of active and past infection. These proteins are potential biomarkers that can be used for the development of diagnostic tools for the detection and characterization of co-infections, or for sero-surveillance markers.

1200

NO CORRELATION BETWEEN PARASITEMIA AND IGG ANTIBODY RESPONSE AGAINST *PLASMODIUM FALCIPARUM* GLUTAMATE-RICH PROTEIN (GLURP-R2) IN SERUM SAMPLES OF PATIENTS FROM IQUITOS, LORETO

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The highly antigenic *Plasmodium falciparum* GLURP-R2 protein is expressed in all stages of the parasite life cycle in human. It is considered as an important vaccine candidate antigen because its interaction with human IgG may play an important role in the development of clinical immunity. The aim of this study was to evaluate the IgG antibody response induced by GLURP R2 antigen in serum samples of patients infected with *P. falciparum* by indirect ELISA. Serum samples from 47 patients, between 9 and 63 years-old, mostly adults, infected with *P. falciparum*, were collected mainly in San Juan and Atalaya districts (province of Maynas), department of Loreto. All samples were positive by PCR and microscopy. Most patients from Atalaya community were asymptomatic, who showed low levels of parasitemia (from 24 to 7477 parasites/μL), while other communities showed higher levels of parasitemia (from 2162 to 61185 parasites/μL). Eight samples of people without any history of malaria disease were used as negative controls. Serum from patients infected with *P. vivax* was used to confirm the specificity of the assay. The cutoff value was calculated using the mean Optical density (OD) plus three standard deviations of negative control group. 87.23% (41/47) and 12.77% (6/47) were seropositive and seronegative to GLURP R2, respectively. The statistical significance of the correlation between parasitemia and IgG response level in both seropositive and seronegative groups was calculated with 95% of confidence ($p<0.05$). There was a weak inverse correlation between IgG response versus Log (parasites/μL) ($R^2=0.303$) for the seropositive group and a direct correlation for the seronegative group ($R^2=0.7806$). In addition, there was no correlation between the IgG response and parasites/μL neither with age or sex of the patient. In conclusion, the absence of significant correlations found shows that the immune response is influenced by other factors either intrinsic or extrinsic to the patient and that GLURP would not be a good vaccine candidate applicable to this region.

1201

PLASMODIUM FALCIPARUM DRUG RESISTANCE MOLECULAR MARKERS UNDER INTERMITTENT PREVENTIVE THERAPY WITH DIHYDROARTEMISININ-PIPERAQUINE (DP) VS. AMODIAQUINE-SULFADOXINE/PYRIMETHAMINE (AQ-SP) IN BURKINA FASO

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Single nucleotide polymorphisms (SNPs) in the *Plasmodium falciparum* pfcrt, pfmdr1, pfdhfr and pfdhps genes are associated with decreased response to aminoquinoline and antifolate antimalarials and have been shown to be selected by use of these drugs. The degree of selection by intermittent preventive therapy (IPT) regimens is unknown. We assessed the baseline prevalence and selection of common SNPs by IPTc in children in Bobo-Dioulasso, Burkina Faso. We studied 1500 children (aged 3-59 months) randomized to receive monthly dihydroartemisinin-piperaquine (DP) or amodiaquine-sulfadoxine/pyrimethamine (AQ/SP) for 3 months during the malaria transmission season in 2009. From random samples of 120 children for each arm of the study and for 120 of 250 untreated controls we evaluated the prevalence of key resistance-mediating SNPs. We then assessed the prevalence of the same SNPs in samples collected in November, 1 month after the third dose of IPTc. Before therapy malaria prevalence was 52.2% (188/360) based on microscopy and 66.67% (240/360) measured by PCR. Prevalences of SNPs were 68.5% (178/260) for Pfcrt 76T; 29.1% (75/258), 58.5% (151/258) and 7.70% (20/260) for Pfmdr1 86Y, 184F and 1246Y, respectively; 58.1% (151/260), 54.8% (143/261), and 55.0% (143/260) for Pfdhfr 51I, 59R and 108N, respectively; and 35.1% (91/259) and 56.8% (147/259) for Pfdhps 436S and 437G. After three monthly IPTc, AQ-SP selected significantly for mutant sequence pfcrt 76T, pfdhfr 59R, 108N and triple mutant 51I, 59R and 108N. DP did not select for known polymorphisms associated with aminoquinoline and antifolate resistance. Our result indicated that IPTc with AQ-SP selected for polymorphisms linked to resistance to AQ and SP probably because of increasing use of these drug. IPTc with DP do not select for known polymorphisms associated with drug resistance. DP may therefore be an excellent alternative for malaria prevention in children in Burkina. Nevertheless, further investigations are needed to confirm this absence of resistant parasite selection following IPTc with DP.

1202

IDENTIFICATION OF A KUPFFER CELL RECEPTOR FOR *PLASMODIUM* SPOROZOITE RECOGNITION

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After inoculation by the bite of an infected mosquito, the *Plasmodium* sporozoite enters the blood stream and infects the liver with unique specificity. To establish a productive hepatocyte infection sporozoites must find and traverse a Kupffer cell, a macrophage-like cell that lines the liver sinusoids. Using a phage display library we identified the P39 peptide that appears to mimic a sporozoite ligand for Kupffer cell recognition. Importantly either preincubation of rat Kupffer cells with P39 peptide or preincubation of *P. berghei* sporozoite with an anti-P39 antibody, inhibits sporozoite entrance into Kupffer cells. We determined that the P39 peptide binds specifically to a ~110 kDa Kupffer cell membrane protein and hypothesize that this protein acts as a sporozoite receptor for Kupffer cell traversal.

1203

CONTRASTING PATTERNS OF SELECTION ON THE ORTHOLOGOUS GENES ENCODING MEROZOITE SURFACE PROTEINS 4 (MSP-4) AND 5 (MSP-5) IN *PLASMODIUM* SPP.

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Merozoites are the invasive form of the malarial blood-stage life-cycle, exposing the merozoite surface proteins (MSPs) on their surface that are involved in initial attachment to the erythrocytes. Given the role of these proteins during the invasion of the red blood cell (RBC), several of them have been considered potential vaccine candidates. Among the MSPs, Merozoite surface protein 4 (MSP-4) and 5 (MSP-5) have received attention since these proteins share crucial structural features. They are glycosylphosphatidylinositol (GPI)-anchored integral membrane proteins with one epidermal growth factor-like domain (EGF) at the C-terminal. In addition, the genes encoding MSP-4 and MSP-5 are closely linked on the genome downstream from the gene encoding the highly conserved enzyme adenylosuccinate lyase (ASL). A single protein (MSP4/5) considered similar to both proteins has been identified in the three rodent malaria species; such a gene has lead to the hypothesis that MSP-4 and MSP-5 originated as a result of an early duplication event. In this study, we investigated the genetic diversity of orthologous genes encoding the MSP-4 and MSP-5 among *Plasmodium* species found in non-human primates that are closely related to *P. vivax*. We also evaluate the hypothesis that these genes are the result of an early duplication event during the evolution of *Plasmodium* in mammals. Overall, we found contrasting patterns of selection acting in genes encoding MSP-4 and MSP-5 in *P. vivax* and related species; MSP-5 orthologs are twice as polymorphic as MSP-4. In addition, we found that the polymorphism in MSP-4 in all *Plasmodium* species included in this study appears to be neutral. In contrast, we found evidence suggesting that MSP-5 in *P. vivax*, *P. cynomolgi* and *P. inui* is under positive selection. Our results reveal that exon I exhibits significant more non-synonymous than synonymous substitutions, confirming previous reports in *P. vivax*. This finding suggests that MSP-5 may be under selective pressure by the immune system across different species of primates including humans.

1204

EXTERNAL QUALITY ASSURANCE PROGRAM FOR *PLASMODIUM FALCIPARUM* RECRUDESCENCE-REINFECTION GENOTYPING IN ANTIMALARIAL DRUG EFFICACY STUDIES

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The authors present this on behalf of the Molecular Surveillance Network for Malaria Drug Resistance in the Greater Mekong Subregion.

The Molecular Surveillance Network is a collaborative effort aiming to strengthen regional and global malaria control and elimination programs by improving quality and comprehensiveness of surveillance for drug resistance and efficacy. The Molecular Surveillance Network partnership includes national malaria control programs of countries in the Greater Mekong Subregion (Cambodia, China, Lao PDR, Myanmar, Thailand, Vietnam) and supports molecular laboratories performing *msp1*, *msp2*, and *glurp* genotyping to distinguish recrudescence from reinfection (RvR) in therapeutic antimalarial drug efficacy studies. Because non-kit-based assays such as RvR testing are difficult to standardize, wide discrepancy can be observed in test results. Factors contributing to this variation include laboratory-laboratory variations in equipment, reagents, supplies and procedures, and the subjective nature of result interpretation, here size-scoring bands on agarose gels. Proficiency testing (PT), an important component of external quality assurance, assesses participants' ability to obtain true results for a set of samples. The Molecular Surveillance Network PT program is a voluntary, confidential testing scheme open to laboratories performing RvR testing on dried blood spot samples. PT panels consist of paired dried blood spots corresponding to pre-treatment (day 0) and post-treatment initiation (day of recurrent parasitemia) samples. Panels are incorporated into routine testing and results are sent to the PT program's organizers for feedback. The PT program was pilot-tested in four laboratories prior to a regional RvR training workshop. Elements of non-conformity included absence of control samples, failure to include gel photos for interpretation and incomplete labeling of results. A post-workshop PT round involving five laboratories resulted in notable improvements in standardization of procedures, use of controls and labeling of samples. Although PT is most powerful when used for quantitative tests with statistically significant numbers of participants, we show that a qualitative, small-scale pilot program for a non-kit-based molecular assay can result in discernable quality improvements.

1205

CHARACTERIZATION OF PFNPC1, A PUTATIVE LIPID TRANSPORTER IN *PLASMODIUM FALCIPARUM*

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During the blood stage of the *Plasmodium* life cycle, the malaria parasite replicates within the erythrocyte, giving rise to 8-32 daughter merozoites. This expansion necessitates large amounts of fatty acids to supply membranes to the newly formed daughter cells. In theory, these fatty acids can either be synthesized by the parasite or acquired from the host. However, recent reports have demonstrated that parasites lacking key enzymes in the fatty acid synthesis pathway have no defect in replication during the blood stage. This suggests that fatty acid acquisition pathways are essential for asexual blood stage development. Indeed, it has been demonstrated that *P. falciparum* requires exogenous sources of both palmitic and oleic acid during blood stage growth. We have initiated studies to define the mechanisms by which fatty acids are imported into intra-erythrocytic parasites. Our studies focus on the role of the *Plasmodium* Niemann-Pick C1 protein homologue, PfNPC1 and its potential role in lipid import. PfNPC1, like its mammalian homologue, NPC1, consists of a sterol sensing domain, a "patched" domain and three large loops. PfNPC1 is expressed during the ring and early trophozoite stage. Fluorescence microscopy of parasites expressing C-terminal GFP-tagged PfNPC1 reveal that this protein is localized to the parasitophorous vacuole, a location that would facilitate the import of host-derived fatty acids. Immuno-electron microscopy is being used to dissect the precise membrane on which this protein resides. Attempts to generate a PfNPC1 knock out have been unsuccessful, suggesting that the protein has an essential function during the blood stage. Ongoing studies aim to elucidate the function of this protein using a conditional knock-down system.

1206

ANALYSIS OF FIELD ISOLATES FROM A CHRONIC *PLASMODIUM FALCIPARUM* INFECTION SUGGESTS THAT VARIANT SURFACE ANTIGENS ARE NOT EXCLUSIVELY COMPOSED OF PFEMP1

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Antigenic variation of variant surface antigens (VSA) enables *Plasmodium falciparum* to establish chronic infections. *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) are suggested to be the major cause of antigenic variation. PfEMP1 is encoded by the multicopy *var* gene family. We have shown that *var* gene transcription in the 3D7 genome strain and in field isolates is biased towards central UpsC *var* genes (Enderes et al. submitted). This raises the question how *P. falciparum* escapes the immune response if it constantly expresses an individual *var* locus. Here we employ parasites and sera from an asymptomatically infected individual to investigate the determinants of antigenic variation. We used shotgun cloning to characterize the *var* gene repertoire at different time points of the infection. Fluorescence activated cell sorting (FACS) was employed to characterize the humoral immune response. To determine individual targets of the antibody response we generated PfEMP1 knock-down parasites in field isolates as well as in NF54 laboratory clones. In these parasites drug pressure removes PfEMP1 from the erythrocyte surface. CD36 receptor binding was used to select for PfEMP1 expression in all parasite lines.The *var* gene repertoire was identical at all time points of the infection, underscoring the parasites ability to evade the human immune response. FACS with sera of the infected individual displayed a strong signal in culture adapted field parasites but not in NF54. However, selection for CD36 binding induced a strong FACS signal in NF54 parasites suggesting crossreactivity with VSA. Application of drug pressure to transgenic NF54 parasites removed this signal, indicating that these antibodies are directed against PfEMP1. Surprisingly, drug pressure had no effect on the FACS signal of transgenic field isolates. This suggests that a large part of the epitopes on these field isolates are not PfEMP1. Taken together our data suggest that antigenic variation is not exclusively mediated by PfEMP1. Transgenic field isolates may provide new insights into the mechanisms mediating immunity to *P. falciparum* malaria.

1207

POPULATION GENETIC INFERENCES OF *PLASMODIUM FALCIPARUM* BASED ON FULLY SEQUENCED GENOMES FROM SENEGAL

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Malaria is a deadly disease that causes nearly one million deaths each year. Understanding the demographic history of the malaria parasite *Plasmodium falciparum* and the genetic basis of its adaptations to antimalarial treatments and the human immune system is important for developing methods to control and eradicate malaria. To study the demographic history and identify genes under selection more efficiently, we sequenced the complete genomes of 25 cultured *P. falciparum* isolates from three cities in Senegal. Based on genetic diversity of the genome sequences, we estimate the long-term effective population size to be approximately 100,000 and show that there is no significant population structure within Senegal. We also estimate a major population expansion of the parasite population approximately 550,000-770,000 years ago. By using the results on demographic history as a null model, the sequences also reveal candidate genes under selection, including pfcrt and dhfr. Moreover, the rates of decay of linkage disequilibrium are fast, indicating the potential of fine-scale genetic mapping in *P. falciparum*.

1208

VARIATION WITHIN THE TOLL-LIKE RECEPTOR-9 (TLR-9) GENE PROMOTER (-1486T/C) IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO PEDIATRIC SEVERE MALARIAL ANEMIA AND FUNCTIONAL CHANGES IN CIRCULATING IFN-Γ

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Plasmodium falciparum malaria is one of the leading causes of infectious disease burden in the world. In holoendemic *P. falciparum* transmission areas, such as western Kenya, severe malarial anemia [SMA, hemoglobin (Hb)<5.0g/dL] results in high rates of pediatric morbidity and mortality. Since Toll-like receptors (TLRs) affect innate and adaptive immune responses, the functional roles of polymorphic variants within the TLR-9 gene in conditioning susceptibility to SMA were investigated. Specifically, the relationship between the TLR-9 variant (-1486T/C, rs187084) and susceptibility to SMA (Hb<5.0 g/dL, any density parasitemia) was investigated in children (n=468) with *falciparum* malaria from a *P. falciparum* holoendemic transmission region in western Kenya. Hematological and parasitological profiles were determined in all study participants. TLR-9 -1486T/C genotypes were determined using TaqMan 5' allele discrimination assay. Circulating interferon (IFN)-γ levels were measured using Biosource™ hu-multiplex inflammatory profile. Frequencies of the -1486TT, TC and CC were 54.4%, 30.7%, and 14.9%, respectively. Multivariate logistic regression analyses controlling for potential confounders demonstrated that homozygous C individuals (OR; 1.68, 95% CI, 1.02-2.77; *P*=0.041) were associated with increased susceptibility to SMA relative to TT individuals. In addition, carriers of the CC genotype had significantly higher circulating IFN-γ levels relative to TT (*P*=0.046). Findings presented here demonstrate that variation in TLR-9 at -1486 is associated with increased susceptibility to SMA and functional changes in circulating IFN-γ levels.

1209

HIGH-DENSITY GENOTYPE ANALYSIS OF A DEEP AFRICAN SAMPLE OF *PLASMODIUM FALCIPARUM*

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We have used a high-density SNP genotyping array (querying 17,000 sites) to examine population structure and differential evidence of natural selection in a set of 93 *P. falciparum* samples from Senegal and the Gambia. After correcting for a small but measurable effect from sample preparation (culture-adapted vs. non-culture-adapted parasites), we find no statistically significant difference in allele frequencies between the two countries, despite different histories of drug use. This suggests that regional data collection should be adequate for genome-wide association studies on this scale. We also examined linkage disequilibrium and find considerable variation across the genome, only some of which can be explained by previously identified selective sweeps. We are continuing our

analysis, including long-range haplotype tests for positive selection, to determine whether these regions represent additional sweeps or areas of reduced recombination.

1210

INVASION OF *PLASMODIUM FALCIPARUM* FIELD ISOLATES FROM SOUTH AMERICA: PHENOTYPIC AND GENOTYPIC ANALYSES

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Invasion of RBCs by *Plasmodium falciparum* involves multiple pathways including those utilizing ligands of the Erythrocyte-Binding Ligand (EBL) and the Reticulocyte-Binding protein homolog (PfRh) families. The invasion of 20 South American (SA) field isolates from Colombia (Antioquia), Peru (Iquitos) and Brazil (Pará) was studied. Seven different invasion profiles were found, one of which is independent of neuraminidase (N), trypsin (T) and chymotrypsin (C) sensitive receptors (NrTrCr), and which was not previously reported. This pathway was used predominantly by Colombian and Peruvian field isolates with varying levels of resistance to the three enzyme treatments (58-93%). Regrettably, majority of other invasion studies did not examine the chymotrypsin treated RBCs for their invasion profile classification. However, even when only the use of the NrTr invasion pathway was compared between the SA isolates and those studied previously, it appeared that 46% of the SA isolates use this pathway in contrast to <5% by African and Brazilian (Mato Grosso) isolates. The use of chymotrypsin treated RBCs allowed us to evaluate the involvement of GPB, and the unknown receptors of EBA-181, PfRh2b and PfRh4 in the alternative invasion pathways of the SA isolates, and which appeared to be more predominant in the Brazilian isolates (5/7). The specific dependence on GPB for invasion was further estimated by using GPB-negative RBCs and the differential use of this receptor vs. the other chymotrypsin sensitive receptors will be presented. Two distinct dominant clusters of invasion profiles were found in SA field isolates: NrTsCs in Brazil, and NrTrCr in Colombia and Peru, both of which are different of those present in Africa, and in part more similar to the Indian field isolates. When the polymorphic variants of the PfRh and EBA-181 and EBL-1 ligands were compared to lab strains and the Mato Grosso field isolates, we found some novel variants in the Peruvian and Colombian field isolates. The association between ligand polymorphisms and the differing invasion pathways used by the SA parasites will be discussed.

1211

USING CF11 CELLULOSE COLUMNS TO QUICKLY, INEXPENSIVELY AND EFFECTIVELY REMOVE HUMAN DNA FROM *PLASMODIUM FALCIPARUM*-INFECTED WHOLE BLOOD SAMPLES

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Next-generation Illumina® sequencing of *Plasmodium* genomes requires depletion of human DNA from parasitized whole blood samples prior to extraction, storage and shipping of DNA to sequencing facilities. The most effective method currently in use is a two-step procedure to deplete leukocytes: centrifugation using density gradient media followed by gravity filtration through expensive, commercially-available columns. This method is not easily implemented in studies collecting hundreds of samples, processing samples for multiple laboratory analyses simultaneously, or lacking capacity for refrigerated centrifugation. Inexpensive syringes hand-packed with CF11 cellulose powder were recently shown to improve ex vivo cultivation of *Plasmodium vivax* obtained from parasitized whole blood samples, as reported previously. We have adapted this procedure to isolate *P. falciparum* DNA from *in vitro* cultured parasites and parasitized whole blood samples obtained ex vivo from Cambodian patients with malaria. Using this method to process blood samples of at least 2 mL and containing at least 10,000 parasites per microliter, we reliably produced 500 nanograms of parasite DNA with less than 30% human DNA contamination. This sample profile is comparable to that obtained by the two-step method and falls well within the current quality control requirements for Illumina® sequencing. In addition, we have validated a centrifuge-free version of the CF11 filtration method to isolate *P. falciparum* DNA at remote and minimally-equipped sites in malaria-endemic areas.

1212

GENETIC DIVERSITY IN *PLASMODIUM FALCIPARUM* MSP GENE FOR 7 DAYS POST-TREATMENT CHARACTERIZE TREATMENT FAILURES IN AN ARTESUNATE MONO-THERAPY TRIAL IN WESTERN CAMBODIA

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Diversity in the *Plasmodium falciparum* genes encoding merozoite surface proteins (*msp) msp1 and msp2*, and *glurp*, has implications for the epidemiology of malaria and the efficacy of malaria drugs. The WHO standard for determining whether a treatment failure is a recrudescence or a new infection uses matched samples from baseline (D_0) and day of failure (D_f). However, in a region of emerging drug resistance, the proportion of parasite at D_0 susceptible to drug may be large and mask a

minute proportion of parasites that are drug resistant. We hypothesized that a small population of resistant parasites may escape drug therapy undetected and reemerge later at the time of treatment failure. In this scenario, a specimen from Day 3 of treatment would better represent parasites that are more resistant to drug and that may actually lead to treatment failure. In a randomized study conducted in an area of emerging artemisinin resistance in western Cambodia during 2008-2009 the efficacy of 7-day courses of artesunate monotherapy for the treatment of uncomplicated *falciparum* malaria were assessed. Samples for nested PCR were collected pre-treatment, on days 2, 3, 4, 5 and 6 of treatment, and on the day of failure (D_f). Patterns of allelic diversity of *msp* and *glurp* were used to distinguish between recrudescence and reinfection by nested PCR. 143 patients were enrolled of who 10 were classified as late treatment failures, 2 as reinfection and 8 recrudescent. A high proportion of isolates from recrudescent subjects showed multiple *msp* allelic types on D_0 and D_f, consistent with a heterogeneous *falciparum* infection. For some subjects, in comparison with D_0, some alleles disappeared by day 3, and re-appeared on D_f. In conclusion, for assessing re-infection and recrudescence in malaria treatment trials, the inclusion of *msp* allelic analysis on post-treatment days 2 through 7, especially day 3, may be a useful addition to the current WHO standard of D_0 and D_f for characterizing allelic distribution.

1213

APPLICATION OF NEXT GENERATION SEQUENCING TO SEARCH FOR ALLELE-SPECIFIC IMMUNITY IN AN RTS,S CLINICAL TRIAL IN MOZAMBIQUE?

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Most candidate malaria vaccines, including the RTS,S vaccine that is now undergoing Phase III trials in Africa, are subunit protein vaccines based on highly polymorphic loci. The goal of this study is to understand the impact of vaccination on the diversity of the parasite population in individual patients. The underlying hypothesis is that successful vaccination is allele-specific and we have tested this hypothesis by comparing parasite populations in RTS,S vaccinated versus control vaccinated individuals. Specifically, we have analyzed the association between parasite diversity at the CSP locus, the antigen target of the RTS,S vaccine, and vaccination in patients who contracted infection during a Phase IIb RTS,S trial conducted in Mozambique. One of the key technical developments necessary to conduct this work is the ability to both detect and quantify the parasite populations within a single patient. To achieve this goal, we developed a new genotyping approach that utilizes next generation sequencing to recover CSP haplotypes in both mixed and single clone infections. Haplotypes capture complex interactions between polymorphisms and are directly associated with protein production and parasite fitness. The sequenced reads were filtered to remove error-prone and misaligned reads and then clustered by similarity into haplotypes. Using model-based filters, we identified and removed SNP errors and chimeras that arise during sample preparation and sequencing and further improved the accuracy of haplotype identification. This approach successfully recovers rare haplotypes (at a frequency of 1%) and yields a sensitive measurement of multiplicity of infection (MOI). The approach has been validated in *in vitro* mixtures of parasites. We assessed whether the parasite populations were different between RTS,S-vaccinated and comparator-vaccinated subjects with respect to specific haplotypes and MOI. Additionally, we re-visited the Enosse et al (2006) analysis to assess whether the increased efficacy of the RTS,S vaccine against severe disease could be attributed to a decrease in MOI. This analysis strategy should prove useful for evaluating allele-specific efficacy in the context of other malaria vaccine trials.

1214

TRANSMISSION BLOCKING ACTIVITY OF ANTIBODIES RAISED AGAINST A PFS25- BASED VACCINE DERIVED FROM NF54 SEQUENCE AGAINST FIELD ISOLATES FROM THAILAND

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Eradication of malaria is possible by the interruption of local mosquito borne malaria transmission and is the end goal in the fight against the disease. Transmission-blocking vaccines (TBVs) that target the sexual stage react with the ookinete surface proteins of malaria parasites within the mosquito midgut, which will contribute to elimination of the disease by blocking the parasite transmission. Pfs25 is a lead TBV candidate, and a Pfs25-based vaccine, Pfs25/ISA51 has been tested in a Phase 1 trial. The vaccine was produced using the Pfs25 sequence from NF54 isolate. Since only a limited sequence polymorphism was reported for this gene, we hypothesized that anti-Pfs25 antibodies induced by this vaccine will have transmission blocking activity against most, if not all, field isolates. To test this hypothesis, we evaluated transmission blocking activities of anti-Pfs25 plasma from the Pfs25/ISA51 trial against parasites in blood of *Plasmodium falciparum* infected patients in Thailand. Normal human Plasma was used as controls. The blood was drawn from each patient and was first tested for transmission blocking activity by membrane feeding assay in triplicates. In parallel, blood samples from these patients were spotted on filter papers for sequencing of Pfs25 genes and for genotyping analysis to determine the independent origin of the parasites. Despite the different genetic background, the Pfs25 sequences from these parasites are identical. The transmission blocking activities of the plasma against these parasites in different blood samples are comparable. Percent reduction in oocyst count in membrane feed assay, when immunized plasma compared with normal plasma is highly significant ($P<0.0001$).

1215

CLINICAL TRIAL OF THE SANARIA® PFSPZ VACCINE VIA THE INTRAVENOUS ROUTE - RATIONALE, PLANS AND PROGRESS

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Immunization by the bites of mosquitoes infected with radiation-attenuated *Plasmodium falciparum* sporozoites remains the most effective method for inducing sustained, high-level protective immunity in humans not treated with anti-malarials. To advance this concept, the *Plasmodium falciparum* Sporozoite (PfSPZ) Vaccine, comprising metabolically-active, non-replicating, purified, aseptic, cryopreserved parasites, has been developed. In the first human trial of the PfSPZ Vaccine, immunization of healthy malaria-naïve volunteers by the subcutaneous (SC) and intradermal (ID) routes was safe and well-tolerated. However, both immunogenicity and protective efficacy were suboptimal. Recent experiments in mice, rabbits, and especially non-human primates (NHPs) demonstrate that the PfSPZ Vaccine is highly potent and that immunogenicity and protective efficacy are far superior when administered intravenously (IV) as compared to SC or ID. In NHP, high levels of SPZ specific CD8+/IFN-g producing cells

were detected in the livers several months after a series of IV but not SC immunizations. *In vitro* data demonstrate that irradiated, aseptic, purified, cryopreserved PfSPZ can invade NHP hepatocytes, providing a potential explanation for such potent responses. Furthermore, administration of labeled SPZ in mice confirm substantially greater distribution of the vaccine to the liver after IV than after SC administration. Together, these animal studies provided the rationale for assessing IV administration of the PfSPZ Vaccine in a Phase 1 clinical trial with experimental challenge. This dose escalation trial is designed to maximize volunteer safety and to provide 1) a clinical proof of principle, 2) a foundation for a clinical development plan leading to licensure of IV-administered vaccine for targeted market segments and 3) a benchmark for development of a non-IV parenteral mode of administration.

1216

VACCINE CANDIDATE IDENTIFICATION FOR PEDIATRIC *FALCIPARUM* MALARIA

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Plasmodium falciparum remains a leading cause of morbidity and mortality in developing countries and vaccines for this parasite are urgently needed. Human residents of endemic areas develop protective immunity that limits parasitemia and disease, and naturally acquired human immunity provides an attractive model for novel vaccine antigen identification. As part of the MOMS project, 785 Tanzanian children living in an area of intense malaria transmission were enrolled at birth, and intensively monitored for parasitemia and clinical illness for up to 3 yrs, with an average of 47-blood smears/child. We identified resistant (n=10) and susceptible (n=10) children based on the results of monthly blood smears obtained from the age of 2 to 3 yrs with matching for potential confounders. Using a differential library screening approach, we identified parasite genes that encode proteins uniquely recognized by plasma pooled from resistant, but not susceptible children. We characterized these candidates with western blot and immunolocalization assays and validated them with independent selections of plasma and with growth inhibition assays. We screened 750,000 clones and identified 3 clones uniquely recognized by resistant but not susceptible plasma. These encoded MSP-7, and hypothetical proteins on chromosomes 10 and 11. We expressed and purified clone 10 and generated anti-sera which, in accordance with *in silico* predictions, recognized a 244 kDa antigen in *P. falciparum* infected, but not uninfected RBCs. In growth inhibition assays, anti-clone 2 anti-sera inhibited parasite growth by 48-63% in several parasite strains. In an ELISA assay using independent selections of resistant (n=11) and susceptible (n=14) plasmas, resistant individuals had 4 fold higher antibody levels to clone 2 proteins compared to susceptible individuals. In conclusion, our differential screening approach identified several novel vaccine candidates and we are currently evaluating the relationship between antibody levels to clone 2 and resistance to infection and disease in the entire birth cohort.

1217

A NEW MALARIA EXPERIMENTAL CHALLENGE SYSTEM: INFECTION OF VOLUNTEERS BY THE BITES OF ASEPTIC *ANOPHELES STEPHENSI* MOSQUITOES INFECTED WITH *PLASMODIUM FALCIPARUM* (NF54) SPOROZOITES

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Experimental malaria sporozoite challenge is an essential component of the vaccine development plan for malaria vaccine candidates targeting pre-erythrocytic stages of the parasite. The current challenge model requires the bites of five infected mosquitoes raised in traditional insectaries to consistently induce malaria. We previously reported on an improved malaria challenge system using the bites of one, three or five aseptically-raised mosquitoes in compliance with cGMP and demonstrated that the aseptic model is safe, associated with a precise prepatent period, and transmitted malaria to all six participants bitten by three *Anopheles stephensi* mosquitoes. As a follow-up study, we evaluated the aseptic model using the bites of three mosquitoes in nineteen additional malaria-naïve adults. In total, twenty-five adults aged 18-40 years (mean= 30 years) were bitten by three A. stephensi mosquitoes infected with the NF54 strain of chloroquine-sensitive *P. falciparum*. The geometric mean sporozoite count detected in challenge mosquitoes was 36.1 x 10^3 (range 6.0-71.1 x 10^3). All twenty-five participants developed microscopy-confirmed peripheral parasitemia, seventeen (68%) on Day 11 post-challenge. The mean prepatent period was 10.9 days (range 9-14 days). The mean parasitemia at diagnosis was 10.8 parasites/µL (range 2-44). Polymerase chain reaction detected malaria in all participants prior to microscopy (mean 3.4 days, range 2-5). No serious adverse events were encountered. The most common solicited events included headache, chills, myalgia, and fever. The aseptic, cGMP-compliant challenge model using three infected mosquitoes transmitted malaria to 100% of participants. The cGMP system provides reliable infection and improves the challenge model by establishing a foundation for assessing the infectivity of sporozoites from aseptic mosquitoes after they have been extracted, purified, vialed, cryopreserved, thawed, and administered by needle and syringe.

1218

PHASE 1 STUDY OF THE SAFETY AND IMMUNOGENICITY OF BSAM-2/ALHYDROGEL®+CPG 7909, AN ASEXUAL BLOOD STAGE VACCINE FOR *PLASMODIUM FALCIPARUM* MALARIA IN ADULTS IN MALI

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A single blind, randomized, controlled Phase 1 clinical trial is being conducted to assess the safety and immunogenicity in malaria exposed adults of the *Plasmodium falciparum* blood stage vaccine BSAM-2, containing a four recombinant protein mixture of AMA1 (AMA1-FVO+AMA1-3D7) and $MSP1_{42}$ ($MSP1_{42}$-FVO+$MSP1_{42}$-3D7) /Alhydrogel® with the novel adjuvant CPG 7909. Participants are healthy adults 18-45 years old living in the village of Bancoumana, Mali. Thirty participants

have received 3 doses (Days 0, 56, and 120) of either BSAM-2 or Euvax B/Hepatitis B vaccine and followed actively for 8 months after the last vaccination and passively for an additional of about 2 months. Enrollment and first vaccinations occurred in March and April of 2010. Vaccinations were well tolerated, with related adverse events being mostly mild or moderate injection site reactions. Antibody responses for AMA1 and $MSP1_{42}$ were higher in the group receiving BSAM-2 for all time points after the first vaccination and the differences were statistically significant ($p<0.05$). There was no significant increase in antibody levels 14 days after the third vaccination compared to 14 days after the second vaccination. The incidence rate of clinical malaria was similar between the vaccination and comparator groups. Despite the favorable safety profile and good immunogenicity, no further clinical development of BSAM2/Alhydrogel®+CPG 7909 is currently anticipated.

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OPTIMIZATION OF A MOUSE CHALLENGE MODEL TO EVALUATE THE EFFICACY OF *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN BASED MALARIA VACCINES

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Future improvements in the protective efficacy of Circumsporozoite protein based vaccines will depend on preclinical data comparing delivery platforms and mixed antigen combinations. Transgenic rodent parasites where the native CSP has been replaced with a functional PfCSP gene will be important tools for down-selecting vaccine candidates. We obtained a transgenic rodent parasite line in which the full-length PfCSP gene was inserted into the *Plasmodium berghei* genome, as reported previously. The parasite was adapted to grow at the WRAIR entomology laboratory using serial passages of sporozoite induced and blood induced infections. We confirmed the transgenic nature of the parasite by IFA with species-specific monoclonal antibodies to CS. We observed normal oocyst development, but significantly reduced salivary gland sporozoite burden in mosquitoes. The minimum infective dose of the sporozoites was established and a series of challenge experiments were conducted comparing several PfCSP based protein vaccine candidates. Protection was defined as complete absence of blood stage parasites on day 15 post challenge. Using a challenge model to down-select vaccine candidates can have important implications for improving CSP based vaccine candidates.

1220

A NOVEL TRICK TO CONTROL MALARIA: MANIPULATING THE MOSQUITO INNATE IMMUNE RESPONSE AGAINST *PLASMODIUM* PARASITES TO BLOCK TRANSMISSION

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Malaria is caused by the protozoan parasite *Plasmodium* which is transmitted by the female *Anopheles* mosquito. The parasite must complete its sexual development in the mosquito before it can be transmitted to the human host. The innate immune response of the mosquito considerably hinders the development of the parasite but this is often not sufficient to clear the infection. In natural infection of the mosquito by *Plasmodium*, there has to be a fine balance between the immune response against the parasite and immune pathology which is reportedly detrimental to the health of the mosquito. We have tried to tip this balance in favour of the mosquito's immune system, which will hinder parasite development and reduce malaria transmission. We have used a viral vectored vaccine platform to express candidate antigens, which are components of the mosquito's innate signalling pathways. Mice have been vaccinated and serum used to measure transmission-blocking activity of antibodies generated by immunization using standardized readouts of *in vivo* efficacy and effect on mosquito survival. This novel strategy could be a revolutionary breakthrough as it would not only work against potentially all four malaria species that infect humans, but likely also against some other mosquito-transmitted diseases and could have a major impact in decreasing the burden of vector-borne diseases. We have also used this vaccine platform to screen several leading parasite and mosquito based malaria transmission blocking vaccine candidates The significance of this work is to provide the first and much needed head-to-head assessment of the *in vivo* efficacy of the known leading TBV candidate antigens, as well as look for novel antigens aimed at de-regulating the mosquito's innate immune system in favour of transmission-blocking activity.

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ANTENATAL MALARIA AND HELMINTH INFECTIONS ARE ASSOCIATED WITH IMPAIRED VACCINE EFFICACY IN KENYAN INFANTS

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African pregnant women are often chronically infected with parasites whose soluble products can cross the placenta and prime or induce immunomodulatory responses in the fetus that can persist into infancy and could affect infant immune responses to childhood vaccines. To test this hypothesis we examined the effect of malaria, schistosomiasis, and intestinal parasites in pregnant Kenyan women (n=545) on the development of IgG antibody responses to tetanus, diphtheria, hepatitis B virus, Haemophilus influenzae type B (Hib), and poliovirus in their offspring following vaccination at 6,12,18,24,30 and 36 months of age. Overall 64.2% of the pregnant women were infected with helminths: 46% and 18% with single and multiple infections respectively. 29%, 20%, 15% and 10% were infected with schistosomiasis, hookworm, or malaria respectively. Children of mothers infected with malaria had lower diphtheria titers at 6, 12 and 18 months of age as compared to children of uninfected mothers ($P<0.01$-0.0009 at each time point estimated by generalized estimating equations). Similarly, offspring of schistosomiasis-infected versus uninfected women had lower diphtheria titers at 12 and 24 months of age ($P<0.01$). In contrast, offspring of schistosomiasis-infected compared to uninfected women had higher polio titers at 12, 18 and 24 months of age ($P<0.01$ at each time point). Children of mothers infected with 2 or more infections had significantly lower Hib-IgG levels at 12 months of age and higher polio-IgG levels at 18 months of age compared to children of mothers with single infection ($P<0.01$). There was no significant difference in antibody levels to any childhood vaccines in children of mothers infected with hookworm, Trichuris, or other intestinal helminths as compared to children of uninfected mothers. Thus, malaria and chronic helminth infections during pregnancy alters responses antibody responses to childhood vaccines and highlight the importance of national programs to eradicate malaria and helminth infections in pregnant women.

1222

CRY1AC PROTOXIN COADMINISTERED WITH *PLASMODIUM* ANTIGEN SYNERGIZES CATALASE ACTIVITY AND NO LEVELS ON CBA/CA MICE INFECTED WITH *P. BERGHEI* ANKA

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We have shown that Cry1Ac induces protection against *Plasmodium chabaudi* AS and *P. berghei* ANKA infection. In this work, we analyzed whether the coadministration of Cry1Ac protoxin with *P. berghei* ANKA antigen (Ag) potentiates this protection and if oxidative stress is associated to parasite elimination. Groups of CBA/Ca mice were weekly treated with: PBS, protoxin Cry1Ac, Ag plus PBS or Ag plus Cry1Ac (Ag+Cry) during 5 weeks, one day after the last injection, mice were infected with *P. berghei* ANKA. Parasitaemia, body weight and survival were recorded daily. In addition, on day 9 post infection splenic mRNA was isolated retrotranscribed and analysed for IFN-γ using qPCR, nitric oxide serum levels and catalase activity also were studied. Mice treated with Ag increased survival for 5 days while mice injected with Ag+Cry survived 8 days more compared to mice treated with PBS (control group), both groups of mice treated with Ag developed lower parasitaemias and lower spleen index compared to control group, furthermore, IFN-γ mRNA expression was upregulated, which implies that with lower cell proliferation the better parasite elimination was attained. Mice treated with Ag+Cry developed significantly higher levels of NO and catalase specific activity in the spleen compared to control group, all thse results suggest that Cry1Ac protoxin could be a potential adjuvant for a malaria vaccine.

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NOVEL APPROACH FOR THE IDENTIFICATION OF NATURAL IMMUNE BOOSTING TRANSMISSION-BLOCKING VACCINE AGAINST *PLASMODIUM FALCIPARUM*

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Antibodies recognizing the surface of *Plasmodium falciparum* zygotes and ookinetes are thought to be ideal for the immunological interruption of malaria parasite transmission from vertebrate host to mosquito.After primary vaccination, antibody responses to such antigens would be boosted during infection. Such an approach would have a advantage over current lead TBV candidates such as Pfs25 that do not naturally induce immune responses in humans because of very low or lack expression in the human host and/or low antigenicity. Here we propose that the identification of antigens shared between gametocytes,sporozoite and zygotes/ookinetes is a new approach to the development of transmission-blocking vaccines (TBV). We hypothesized that highly expressed ookinete surface proteins of *P. falciparum* that are also expressed by asexuals, gametocytes or sporozoites would boost transmission-blocking antibodies during natural malaria infection. To test this hypothesis, we used existing ookinete proteome data and a *P. falciparum* protein microarray to identify antigens that might boost transmission-blocking activity by being shared among blood and mosquito midgut stages. 110 African patient sera recognized 79 predicted ookinete surface proteins of *P. falciparum* (*P. gallinaceum* ookinete orthologs) on the protein microarray; several ookinete surface proteins were found also to be expressed in gametocyte, sporozoite or asexual blood stage parasites. The hypothetical PF11_0055 gene product contains a predicted thioredoxin-like domain, is highly conserved (79%) in *P. berghei*, is expressed in all stages, and was immunogenic. Vaccination of mice with recombinant E. coli-produced PF11_0055, followed by *P. falciparum* gametocyte lysate, boosted anti-PF11_0055 antibody titer compared to gametocyte lysate alone used as vaccine. In standard membrane feeding assays, antibodies to PF11_0055 antibodies significantly reduced oocyst numbers and infected mosquito prevalence. Another protein, PfCelTOS, a known sporozoite- and ookinete-expressed protein, was found to be abundant in ookinetes and highly immunogenic (spec count: ookinete 84, sporozoite 58; geometric mean titer 6796); polyclonal mice sera effectively blocked oocyst development in *P. falciparum*. This new approach to transmission-blocking vaccine candidate discovery based on systems biology antigen discovery is a promising new direction in malaria vaccinology.

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IMMUNODAMPENING TO OVERCOME DIVERSITY IN THE MALARIAL VACCINE CANDIDATE APICAL MEMBRANE ANTIGEN 1

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Apical membrane antigen 1 (AMA1) is a leading candidate for inclusion in a malaria vaccine however the polymorphic nature of this protein may limit its efficacy. Within AMA1, the highly variant loop Id has been identified as a dominant target of strain-specific, inhibitory antibodies. In this study we aimed to circumvent AMA1 diversity by dampening the immune response to loop Id and enhancing the response to more conserved epitopes. To achieve this, five polymorphic residues in loop Id were mutated to alanine, glycine or serine and initially the corresponding antigens were displayed on the surface of bacteriophage to assess their ability to fold correctly. Reactivity with conformation-sensitive antibodies indicated that glycine substitution compromised formation of the correct disulphide-bonded structure and the glycine mutants were therefore not produced as purified recombinant proteins. Since phage-based assays indicated that the alanine and serine mutants were correctly folded, these variants were expressed in *E. coli*, refolded *in vitro* and used to immunize rabbits. Serological analyses indicated that immunization with a single mutated form of AMA1 was sufficient to increase the cross-reactive immune response. Furthermore, combining engineered forms of AMA1 derived from two different alleles was more effective at broadening the immune response than combining the two corresponding wild type antigens. This suggests that inclusion of a mutated form of AMA1 in a malaria vaccine may reduce the number of variants required to induce a sufficiently broad immune response. We are currently expanding this study to determine which combination of wild type and/or mutant AMA1 offers the most promise for protection from diverse *Plasmodium falciparum* genotypes.

1225

LANDSCAPE OF RESPONSIBILITY: EVOLVING OF ROLES AND RESPONSIBILITIES FOR COMMUNITIES AND INSTITUTIONS IN A LARVAL CONTROL PROGRAM FOR MALARIA PREVENTION IN URBAN DAR ES SALAAM, TANZANIA

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In the first half of the 20th century, targeting the larvae of mosquitoes was regarded as a practical means to reduce malaria in cities. However, because it is labour intensive and demands considerable manpower, entomological expertise as well as institutional oversight, this approach fell out of favour for decades and is only recently being reconsidered.

This analysis describes a community-based programme for larval control of malaria vector mosquitoes in urban Dar es Salaam, Tanzania, as an example of how scientific research and public health governance can be mutually configured in a contemporary African city. Initiated by the Dar es Salaam City Council, the Urban Malaria Control Program (UMCP) was designed to investigate the effectiveness of community-based systems for applying microbial larvicides, to aquatic breeding habitats in reducing the prevalence of malaria. The UMCP aims to demonstrate the operational feasibility of integrating larval control into routine municipal services, relying exclusively for its implementation on community-owned resource personnel (CORPs). The UMCP was therefore, designed to transform Dar es Salaam into both a venue of local management and a site of knowledge production. Drawing on ethnographic and historical resources, we consider the socio-technical practices these parallel transformations entail. In particular, we are concerned with how 'participation in' and 'responsibility for' larval control is inter-articulated through scientific protocols, development practices, and the specific political history of Tanzania. Through an analysis of the activities of the CORPs, we suggest that public health governance should be understood within a series of partial and spatially-bound relationships: between residents, local government from neighbourhood to city level research institutions and the reproduction traits of specific mosquito species. We conclude that to enable scaling up of a community-based intervention to a sustainably effective programme at city or national level requires, first, attention to the political history of those relationships and, second, an understanding of how responsibility for malaria control and public health more broadly, is best distributed within the simultaneous contexts of a scientific evaluation and a government-led programme.

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PHYSICAL DURABILITY OF TWO TYPES OF LONG-LASTING INSECTICIDAL NETS (LLINS) AFTER TWO YEARS OF USE, MOZAMBIQUE 2008-2010

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Understanding the physical durability (PD) of long-lasting insecticidal nets (LLINs) is critical to guide malaria programs on the frequency of LLIN replacement. We conducted a prospective evaluation of LLIN PD after a distribution campaign in October 2008 in Nampula Province, Mozambique.During the LLIN campaign we tagged 1000 LLINs of two types (polyethylene [PT] and polyester [PS]) at six distribution sites (6000 LLINs tagged). The tagged LLINs were geo-located during a house-to-house survey one month after the campaign and a random sample of households (HHs) was selected. One and two years after the campaign, the selected HHs were surveyed and all tagged LLINs were collected. LLINs were stretched over a frame against a black background and all holes were quantified. The difference in total number of holes by LLIN type and year was analyzed by unadjusted chi-square and the median number of holes and inter-quartile range (IQR) by hole size was analyzed using Wilcoxon rank sum test. One year after distribution 164 out of 210 HHs were interviewed and 148 LLINs were recovered and assessed; 50 of 51 (98%) PT and 73 of 97 (75%) PS had at least one hole ($p<0.0004$). Two years after distribution, 197 out of 240 HHs were interviewed and 163 LLINs were recovered; 58 of 59 (98%) PT and 97 of 104 (93%) PS had at least one hole ($p = 0.15$). The median number and IQR of holes after one and two years of use, respectively, was 18 (9, 33) and 53 (28, 98) for PT and 4 (1, 12) and 15 (5, 45) for PS. For both years, PT had a statistically significant higher number of holes of all sizes compared to PS ($p < 0.0001$). We found significant proportions of LLINs are damaged already by year one, more so for PT than PS. How this damage to LLINs translates into loss of protection against malaria transmission is not yet known. Additional studies are needed to measure the impact of the number and size of holes and physical integrity of the LLINs on malaria transmission to define LLIN failure.

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FREE NET DISTRIBUTION: WILL A HANG-UP CAMPAIGN MAKE AN IMPACT ON USE?

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Insecticide treated nets (ITNs) are highly effective in reducing malaria morbidity and mortality when used appropriately and consistently. The Angolan Ministry of Health (MoH) recently revised its National Strategic Plan for 2011-2015 to expand ITN coverage beyond pregnant women and children under five to universal coverage. An effective method to reach and maintain high net coverage is free distribution campaigns. Post-distribution hang-up campaigns to assist in and ensure utilization of ITNs have been implemented in several sub-Saharan countries. Survey results to evaluate the effectiveness of these campaigns indicate higher use of ITNs. In Angola, the first major free net distribution campaign targeting universal coverage is currently underway (April-August 2011). Africare is implementing the campaign in thirty-two communities in four municipalities in two provinces.176,000 ITNs will be distributed to reach universal coverage in these communities. Door-to-door registration confirms household size, the number of existing ITNs, and the number of additional nets required to ensure each household member has access to an ITN. Vouchers for free ITNs are distributed at the time of the door-to-door registration and are redeemed two weeks later at a central distribution location. Distribution is complemented by community awareness and education activities around malaria prevention and transmission. Activities include demonstrations of how to properly hang and care for ITNs. In two of the four municipalities, a hang-up campaign is being conducted in which community activists visit all households to assist hanging the nets in sleeping spaces. A post-campaign survey to assess ITN coverage and usage is planned for August 2011. Based on interim data collected, a higher use rate is expected in the two municipalities receiving the hang-up campaigns compared to those not receiving this intervention. This campaign is important as it will illuminate important barriers, challenges and opportunities that Angola's MoH can then use to design effective programming to achieve its goal of universal ITN coverage.

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LOW COST REPELLENTS FOR MALARIA PREVENTION IN RURAL AFRICA: THE JURY IS STILL OUT

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Malaria control using Long Lasting Insecticidal Nets (LLINs) is a highly effective strategy for rural Africa. However, there is growing evidence that malaria vectors are switching their feeding behavior to the early evening when people are not under their nets and are available to feed on. A cluster randomized controlled clinical trial was conducted in a village in Southern Tanzania from June 2009 to September 2010 to evaluate the additional protection provided by a 15% deet (di-ethyl toulamide) repellent lotion among LLIN users compared to LLIN users given a lotion with no deet. Consistent repellent use in the early evening may provide protection from clinical malaria episodes transmitted by early evening feeding mosquitoes. However, the power of this study was insufficiently low to draw a firm conclusion from the data. The estimate protective efficacy was 13%, lower that that expected. In order to measure this effect with sufficient power a sample size of more than 5,000 households per arm would be required. The role of repellents in malaria prevention

remains uncertain. Although there were 13% fewer clinical malaria episodes among repellent users compared to the placebo this difference did not reach statistical significance and in order to be sure that repellents are protective a much larger trial would have to be carried out. Repellents were extremely popular and the relief from nuisance biting mosquitoes was a major motivation for their use. They would need to be cheap in order to encourage uptake and strategies such as seasonal promotion prior to peak malaria season could be employed in order to maximize their potential for protection from malaria.

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IMPORTANCE OF SLEEPING ARRANGEMENT TO INCREASE BED NET USE AND REDUCE MALARIA TRANSMISSION

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A previous study found that older children tend to sleep on the living room floor without mosquito nets in villages along Lake Victoria, western Kenya. The study suggested that it is not easy for children to hang a net in a living room. We examined if this situation increases malaria transmission. A total of 849 children less than ten years old were tested for malaria infection using rapid diagnostic tests (RDT). Their caretakers were asked about bed nets use and sleeping arrangement. Of them, 530 children (62.4%) were tested positive. Nearly 70% of them did not sleep on beds, and almost half of them did not use bed nets. Older children more likely slept on the living room floor. Bed net use was lower among older children, and among children who slept on the floor. Children who slept without nets had a higher positive rate for malaria infection. Older children had a higher positive rate. When the analysis was limited to children above five years old, the result of RDT was not significantly correlated with bed net use and sleeping arrangement. The positive rate of older children was 68.7%, while that of younger children was 57.1%. These results suggest that sleeping arrangement is particularly important for younger children to prevent malaria infection.

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SOLAR-POWERED FAN PROVIDES VENTILATION WHILE SLEEPING UNDER INSECTICIDE TREATED BED NETS

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Insecticide-treated bed nets have been shown to reduce transmission of malaria by 50% in numerous endemic regions. However, many recipients are not using their bed nets due to uncomfortably hot conditions while sleeping inside of them. Here we have developed a prototype solar rechargeable fan that can be easily positioned inside the enclosed bed net space to provide ventilation and cool off the occupants. The fan features a self-contained battery pack, motor, switch, and charging circuit that allows the 9 in. long fan assembly to be plugged into the separate solar panel power source. The objective is six hours of exposure to sunlight charges to the battery pack to enable 8 hours of constant operation. The constructed prototype is a proof of concept to show that it is feasible to create a small, efficient solar powered fan. Refinements to the existing prototype will include an alternative battery pack to reduce costs, and design modifications to decrease charging time and to increase air circulation and handling.

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ENTOMOLOGICAL MONITORING OF AN INDOOR RESIDUAL SPRAYING (IRS) PROGRAM IN MALAWI

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A pilot indoor residual spray (IRS) program was initiated in Malawi in 2007 in one district with funding from the President's Malaria Initiative (PMI) and scaled up to six additional districts in 2010. Two insecticides were used, alphacypermethrin (Mokrid), and pirimiphos-methyl (Actellic). Vector abundance, insecticide decay rates and insecticide resistance were monitored to assess impact of the program. Monitoring was carried out in twenty-one villages in all the seven IRS districts. Vector abundance was monitored quarterly in three districts using pyrethrum space spray catches (PSCs) and monthly in four districts sprayed with alphacypermethrin and pirimiphos-methyl. Susceptibility tests were also carried out before spraying in selected villages in all the districts and insecticide decay rates were monitored monthly in two districts following WHO standard techniques. The main malaria vectors prevalent in the IRS districts were *Anopheles gambiae s.l* and *An. funestus s.l.* Spraying with alphacypermethrin reduced the density of *An. gambiae* to almost zero in villages where this species was predominant. There was marked reduction (>50%) in the abundance of *An. funestus* in areas where it exclusively occurred. Use of pirimiphos-methyl reduced the abundance of *An. funestus* by >90% in the areas where this species previously exhibited pyrethroid resistance. Mortality of *An. gambiae* Kisumu strain exposed onto walls sprayed with alphacypermethrin was <80% one month after spraying. On the hand, pirimiphos-methyl residues remained active for two months. Baseline susceptibility tests showed that *An. funestus* was resistant to pyrethroids (approx. 30% resistant) but susceptible to pirimiphos-methyl (100%). *An. gambiae* from Karonga District was susceptible to pyrethroids (100%). As expected, spraying with alphacypermethrin reduced populations of *An. gambiae*. The reduction observed in the population of *An. funestus* was, however, unexpected considering that the species showed resistance to pyrethroids. Use of pirimiphos-methyl resulted in marked reduction in the abundance of a previously known resistant populations of *An. funestus*. Despite these gains, the IRS program in Malawi faced a number of challenges both logistical and biological.

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FIGHTING MALARIA WITH ENGINEERED BACTERIA

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The mosquito midgut plays a key role in the malaria parasites development and subsequent transmission and also provides the habitat for diverse symbiotc bacteria. We are exploring the use of such naturally occurring bacteria as a vehicle to deliver anti-malaria effectors molecules in mosquito midgut. Specifically, we engineered *Pantoea agglomerans*, a bacterium commonly found in the midgut of *Anopheline* mosquitoes, to express a variety of effector molecules. These include 1) Salivary gland and Midgut peptide 1 (SM1), 2) phospholipase A2 (PLA2), 3) *a* single-chain immunotoxin (pbs21:shiva) composed of a single-chain antibody targeting the ookinete surface protein pbs21 and a lytic peptide Shiva-1, 4) a chitinase propeptide (Prochit) that inhibits chitinase and blocks ookinete traversal of the mosquito peritrophic matrix, 5) scorpine, a multifunctional antimicrobial peptide and 6) a *Plasmodium* enolase lysine-

rich inhibitory hexapeptide (Lrmp) that prevents plasminogen binding to the ookinete surface. By using the *E. coli* haemolysin A transport system (HlyA), the corresponding proteins were effectively secreted by transgenic *P. agglomerans* cells and accumulated in the culture media as determined by SDS-PAGE and Western-blot analysis. *In vivo* secretion of SM1 and PLA2 was confirmed by use of immunofluorescent assays that detected the binding of these proteins to mosquito midguts. Importantly, the engineered bacteria efficiently inhibited development of the human malaria *P. falciparum* in mosquitoes. *P. falciparum* oocyst counts were inhibited by 85-98%, depending on the effector gene. Significantly, the ability of mosquitoes to transmit the parasite (prevalence) was decreased by 97% for two of the effector genes (scorpine and $(Lrmp)_4$). Our findings suggest that engineered bacteria may be used to significantly strengthen existing malaria-control strategies.

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SUSTAINABLE SUPPLY CHAINS: LESSONS LEARNED FROM A LONG LASTING INSECTICIDAL NET RECYCLING PILOT PROJECT IN MADAGASCAR

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Distribution, use and timely replacement of long-lasting insecticide treated nets (LLINs) are part of a key malaria prevention strategy in Madagascar, where 5.2 million LNs were distributed from 2005-2007. There is a growing awareness of the potential environmental impact of insecticide-embedded plastic waste from the increased number of LNs, if not disposed of or recycled in an environmentally sound manner. We conducted a pilot project to collect and recycle existing old, expired LNs (oLNs) in conjunction with a mass free LN distribution campaign in November, 2010. Six health districts with an estimated population of 1.6 million were targeted for the pilot where 279,000 bed nets had been distributed in 2007. Health volunteers were trained to educate their communities, using a pre-tested job-aid, to voluntarily bring unwanted oLNs for disposal to the closest campaign community distribution point at the time of collecting their new free LNs. oLNs were collected, transported, sorted, compacted, baled and shipped to a plastics recycling company for processing. Over 22,500 oLNs were collected from 394 out of 489 (81%) community collection points. Of these, 90% were collected post-campaign. Community members were more willing to give up oLNs once the new LNs were installed in homes after the campaign distribution. Families with an insufficient number of new nets, and those using oLNs for other purposes, were reluctant to give up their oLNs. Sites with the most complex transport logistics were less likely to successfully collect oLNs. Post hoc radio messaging was found to be a useful tool to reinforce messages. The cost was $2.72/oLN collected. Costs could be substantially reduced by combining training with other LN distribution campaign preparation activities. LLINs have been successfully recycled and the material is being analyzed and tested for the most appropriate recycling use. In conclusion, collection and recycling of oLNs was found to be acceptable and feasible. Malaria programs and international donors should further explore and implement cost-effective recycling and re-use options.

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BEHAVIOR CHANGE COMMUNICATIONS (BCC) FOR MALARIA CONTROL IN SOUTHEAST NIGERIA

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Nigeria is engaged in a massive nation-wide distribution of long-lasting insecticidal nets (LLIN). LLIN ownership may be the strongest determinant of use, but there is a need for effective, evidence-based interventions to address other key determinants of net use. To inform the development of BCC strategies, The Carter Center added questions about social and behavioral determinants of LLIN use to a survey conducted in Southeast Nigeria in December 2010, prior to mass LLIN distribution campaigns. Preliminary data are presented here from 1290 individuals (43% male, 57% female) in 1192 households of Imo and Ebonyi States (2 LGAs/ state, 17 clusters/ LGA). While 83% of respondents know that malaria is transmitted by mosquitoes, 66% believe that people are only at risk for malaria during the rainy season, and 65% believe that you get malaria from eating certain foods. 72% of respondents listed protection from mosquito bites as the purpose of LLINs, but only 15% mentioned malaria prevention. Heat (15%) and allergies (5%) were rarely mentioned as disadvantages, and 42% said that LLINs have no disadvantages. 81% agreed that LLINs can be hung over any sleeping space, and 90% said LLINs are safe to sleep under. However, only 54% believe that it is safe to hang a net where you store food, and only 2.4% are aware that LLINs do not have to be re-treated. Bed nets have some negative associations: 33% think bed nets are for poor farmers, 39% think nets are "old fashioned," and 27% believe nets are part of a Western plot to reduce African populations. The data suggest that factors other than knowledge or intrinsic characteristics of LLINs may be important determinants of use (such as situational factors, norms and social support). Low literacy 46%) and limited comprehension of languages generally used for malaria communications (English 23%, Pidgin 16%), as well as limited exposure to and widespread distrust of many sources of health information, suggest that home visits conducted by trusted community members may be the most appropriate channel for malaria BCC in these areas.

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USEFULNESS OF FORMATIVE RESEARCH AS RAPID ASSESSMENT TOOL TO GUIDE IMPLEMENTATION OF INSECTICIDE-TREATED CURTAIN INTERVENTION IN IQUITOS, PERU

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As part of a community-randomized trial to evaluate the effectiveness of insecticide-treated curtains (ITC) for dengue prevention in Iquitos, Peru, formative research was conducted to assess rapidly the acceptability of ITCs in the population, and guide the research team on the approach for ITC distribution. Forty-five individuals, aged between 25 and 60 years, participated in five focus groups discussions (FGD) on the topic. After describing the curtains and passing around small ITC samples, in all five groups, all participants felt the curtains would be well accepted by themselves and people in their community. Their comments focused on how the ITC "would be favorable to families" and that it is needed

because "there are many mosquitoes in our community" or because "there is much dengue and hemorrhagic dengue around". Though overall levels of concern were low, the main one expressed related to potential allergic reactions to the ITC, particularly among children. Through the FGD we also assessed the style of curtain that people might prefer (lacey ITC was favored by all over simple bednet style because it was "more elegant"), the colors favored (light colors were preferred for most spaces, except where people would use the curtain for additional privacy), and the number of curtains people might request (median number requested was 5). The information obtained allowed us to obtain an appropriate amount and color combination of ITCs for the initiation of the trial. Also, we developed a tri-fold describing the purpose of the study, the ITCs, and providing information on how to care for the curtains, making sure to incorporate the types of concerns expressed during the focus groups. Formative research allowed us to obtain information in a rapid and cost-effective manner that was useful for the start up of our trial.

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PROMOTION OF UTILIZATION OF INSECTICIDE-TREATED NETS IN A MULTICULTURAL COMMUNITY ALONG THE THAI-MYANMAR BORDER

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This quasi-experimental study was conducted between June 2007 and April 2008, in two villages of Sangkhlaburi District, Kanchanaburi Province, Thailand. It aimed to assess the effectiveness of a health-promotion program to prevent malaria, emphasizing the utilization of insecticide-treated nets (ITN) in a multicultural community. This study applied the PRECEDE-PROCEED model for planning, implementing, and evaluating the program. It adopted four health-promotion strategies--building capacity, establishing partners and building alliances, health communication, and health education. The study was conducted in a community composed of highly diverse ethnic groups living in malaria-transmission areas along national borders. Health-promotion program activities were planned and implemented taking into account the diversity of the target population. Villagers from various ethnic groups were motivated and invited to be health-promotion volunteers. Training workshops were organized for health officers and health-promotion volunteers, to increase their capacity related to the treatment of nets and delivery of health education and health communication. The bilingual materials used for health communication and health education were co-produced by volunteers and the research team. Net re-treatment was organized twice. The effectiveness of the health-promotion program was assessed by comparing program pre- and post-test results. The results showed that the health-promotion program for malaria prevention, emphasizing the utilization of insecticide-treated nets in a multicultural community, did increase appropriate ITN use. The proportion of nets being treated and net users in the intervention group increased significantly (p value=0.00).

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BIODIVERSITY OF MOSQUITOES (*DIPTERA: CULICIDAE*) AND SAND FLIES (*DIPTERA:PHLEBOTOMINAE*) FROM THE NORTHWEST REGION OF LORETO DEPARMENT IN PERU

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From January to March 2009, mosquitoes and sand flies were collected in four villages located on the margens of the Huallaga and Maranon Rivers,in the provinces of Alto Amazonas and Datem del Maraii6n,located in Loreto Department,Peru. Collections were made using CDC light traps,human bait, and back-pack aspira tors in peridomiciliary areas. The entomologic material was kept in liquid nitrogen and transported to the Entomology Lab of NMRCD in Lima, where taxonomic identification was carried by mosquitoes and sandflies. A total of 22,513 mosquitoes were identified: 21,899 (97.27%} Culicinae and 614 (2.71%) Anophelinae. Mosquito capture ra tes were 75.28% using CDC light traps, and 17.27% using human bait, and 7.45% using back-pack aspirators. Throughout the process after collection (transport,storage,taxonomic identification), mosquitoes were preserved in cryovials at a temperature of -80°C. Biodiversity rates of *Anopheles* spp. subgenera *Anopheles, Nyssorhynchus* and *S tethomyia* were determined. *Anopheles (Nyssorhynchus)* spp. had the highest density in all collections. Eleven genera of Culicinae were identified, the *Culex* genus (with two subgenera and about 10 species identified) had the highest number of collected mosquitoes, followed by the genera *Mansonia, Ochlerotatus, Psorophoro* and *Coquillettidia.* The Shannon-Weaver diversity index was high with CDC light traps (H = 1.04), in relation to the other collection methods. In relation to sand flies,113 specimens of the genus *Lutzomyia* (77 females and 36 males) were identified, with 11species and three *Lutzamyia* spp., from which *Lutzomyia (Nyssomyia) antunesi* had the largest number of collections (64 sand flies), followed by *Lutzomyia (Nys.) yuilli yuilli* (14).

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CO-OCCURRENCE PATTERNS OF THE DENGUE VECTOR *AEDES AEGYPTI* AND *AE. MEDIOVITATTUS*, A POTENTIAL NATIVE DENGUE VECTOR IN PUERTO RICO

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Aedes aegypti is implicated in dengue transmission in tropical and subtropical urban areas around the world. *Ae. aegypti* populations are controlled through integrative vector management. However the efficacy of vector control may be undermined by the presence of alternative, competent mosquito species. In Puerto Rico, a native mosquito, *Ae. mediovittatus*, is a competent dengue vector in laboratory settings and it spatially overlaps with *Ae. aegypti*. It has been proposed that *Ae. mediovittatus* may act as a dengue reservoir during interepidemic periods, perpetuating endemic dengue transmission in rural Puerto Rico. Dengue transmission dynamics may therefore be influenced by the spatial overlap of Ae mediovittatus, *Ae. aegypti*, dengue viruses, and humans. We take a landscape epidemiology approach to examine the association between landscape composition and configuration and the distribution of each these Aedes species and their co-occurrence. We used remotely-sensed data from a newly launched satellite to map landscape features at very high spatial resolution. We found that the distribution of *Ae. aegypti* is positively predicted by urban/built-up density and by the number of tree patches, *Ae. mediovittatus* is positively predicted by the number of tree patches, but negatively predicted by large contiguous urban/built-up areas, and both species are predicted by urban/built-up density and the number of tree patches. This analysis provides evidence that landscape composition and configuration is a surrogate for mosquito community composition, and suggests that mapping landscape structure can be used to inform vector control efforts as well as to inform urban planning.

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DIFFERENTIAL EXPRESSION OF *AEDES AEGYPTI* SALIVARY PROTEOME UPON CHIKUNGUNYA VIRUS INFECTION

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Mosquito-borne diseases are excellent examples of emerging and resurging diseases that are significant global public health threats. Chikungunya virus (CHIKV) infection caused an explosive outbreak that infected as many as two million people during 2006 in India and the islands of the Indian Ocean with subsequent spread to other parts of the world. This resurging infection is transmitted primarily by *Aedes aegypti* and *Ae. albopictus*. Saliva of *Ae. aegypti* contains a complex array

of proteins essential for both successful blood feeding and pathogen transmission. Understanding salivary gland protein expression during the extrinsic incubation period of CHIKV infection is important since changes in salivary gland physiology and saliva composition could influence mosquito blood feeding success and virus transmission. CHIKV regulated mosquito salivary proteins could modulate host innate and acquired immune responses at the bite site and systemically, resulting in impaired antiviral effector functions. Using a differential proteomic approach we investigated the differential mosquito salivary protein expression during CHIKV infection. Adult female mosquitoes were fed with either CHIKV infected or uninfected bovine blood using a Hemotek membrane feeding system. Salivary glands were dissected eight days postfeeding, and proteins were extracted in 2D gel buffer. One hundred micrograms of proteins were resolved on a 2D-gel and stained with SYPRO-Ruby stain. Protein spots with a relative difference of greater than two fold, and a p-value less than 0.05 were considered a significant variation. These protein spots were excised, tryptic digested and prepared for MALDI-TOF-TOF and LC-MS-MS analysis. The expression of 22 proteins was found to be up-regulated, while 33 proteins were down-regulated. Among the up-regulated proteins, adenosine deaminase and D7 proteins have been implicated to play a major role in mosquito blood feeding. The D7 proteins belong to the family of arthropod odorant binding proteins, that facilitate blood feeding by binding to biogenic amines. These proteins are believed have anti-hemostatic and anti-inflammatory functions. Interestingly, several of the differentially expressed proteins in the salivary gland induced by CHIKV infection are proteins with unknown functions. This preliminary data establishes that CHIKV modulates mosquito salivary gland protein expression.

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BLOODFEEDING PATTERNS OF *CULEX TARSALIS* AND THE *CX. PIPIENS* COMPLEX IN CALIFORNIA

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West Nile virus (WNV) is a mosquito-borne flavivirus now endemic across several ecological regions in California. These regions are home to a wide diversity of potential avian and mammalian hosts as well *Culex* vector species. Because avian hosts have varying WNV competence, determining the bloodfeeding patterns of the *Culex* vectors is important in understanding the dynamics of virus maintenance as well as incidental transmission to disease-susceptible humans and horses. The bloodfeeding patterns of *Cx. tarsalis* and members of the *Cx. pipiens* complex were investigated from 5 locations spanning over 850km from Northern to Southern California. Nearly 100 different avian, mammalian and reptilian host species were identified from 1,487 bloodmeals using DNA sequence from a portion of the mitochondrial gene, cytochrome c oxidase I (*COI*). *Cx. tarsalis* fed on a higher diversity of hosts and more frequently on non-human mammals than did members of the *Cx. pipiens* complex when collected in the same area. Several WNV competent avian species, including House Finch and House Sparrow, were common bloodmeal sources for both vector species across several ecological regions and could account for WNV maintenance, particularly in urban settings. Highly competent Western Scrub-Jay, Yellow-billed Magpie, and American Crow also were fed upon frequently when available and are likely important amplifying hosts in some areas. The *Cx. pipiens* complex (0.4%) fed more frequently on humans than did *Cx. tarsalis* (0.2%), and horse bloodmeals were only identified from *Cx. tarsalis* (2.3%). Although neither vector species fed frequently on humans or horses in this study, with high vector abundance both species could serve as bridge vectors of WNV in several California regions.

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BARRIERS TO MALARIA ELIMINATION ON THE ISLANDS OF ZANZIBAR

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The islands of Zanzibar are the major focus of a malaria elimination campaign (defined as the reduction to zero of the incidence of locally acquired malaria). It is generally supposed that *P. falciparum* in Zanzibar is vectored by *Anopheles* species that are endophillic, anthropophagic and pyrethroid-susceptible. As a result, the islands have been saturated with permethrin or alphacypermethrin treated LLINs (long lasting insecticide treated nets) and IRS (indoor residual spraying) with lambdacyhalothrin. These campaigns have been extremely effective at reducing the prevalence of malaria to less than 1 percent. It now seems however, that the move to an elimination stage will be complicated by some recent discoveries on the ecology and behaviour of local mosquito populations. Studies conducted on the island of Pemba by the Zanzibar Malaria Control Program during 2010 and 2011, now show that most of the remaining transmission in Pemba is probably being mediated by *An. arabiensis*, and that (as a consequence of the behavioural plasticity of that species, and the high coverage of pyrethroids indoors) the majority of bites are now received out-of-door. This suggests that LLINs and IRS may need to be augmented by other control methods in order to reduce mosquito-human contact further. Moreover, a phenological characterisation of *An. arabiensis* from Pemba have shown these populations to be resistant to all pyrethroids (but susceptible to DDT, malathion and bendiocarb). The magnitude of the resistance is sufficient to markedly reduce mortality in simple bioassays against IRS residues, and used LLINs. This has prompted ZMCP and its partners to implement a change in IRS practice but with so few new vector control interventions available, or even in the pipeline, opportunities to improve upon existing control practices are very limited.

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NOVEL SOLUTIONS FOR THE DETECTION, PREVENTION AND TREATMENT OF VECTOR-BORNE DISEASES

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Florida's Schools of Pharmacy at UF and USF in concert with the UF-Emerging Pathogens Institute (EPI) and USF Center for Drug Discovery and Innovation (CDDI) and Global Health Infectious Disease Research Program (GHIDR) are developing a consortium for novel solutions for the Detection, Prevention and Treatment of Vector Borne Diseases. Vector borne diseases represent a significant health care challenge for Florida (and the tropical world), but there has been little economic incentive for the pharmaceutical industry to develop interventions. Our proposed consortium is critical to catalyze the development of efficient strategies able to solve this regional/global health-care challenge. The proposed consortium will provide a "case study" to introduce the FDA's Critical Path Initiative Development Toolkit to Florida institutions, with a focus on developing powerful scientific and technical methods such as *in vitro*, animal or computer-based predictive models, biomarkers for safety and effectiveness and new clinical evaluation techniques for a streamlined and efficient drug development as well as for establishing new validated

methods of detection and preventions. This new USF-UF consortium will place emphasis on product innovation and translational medicine and allow students and faculty to participate as team members in high profile epidemiological, drug discovery and development projects. Our consortium will "pull" and our Centers and Institutes will "push" the best emerging biomedical and biopharmaceutical technologies in Florida. Resulting infrastructure will facilitate faculty scholarship and intellectual engagement between our Universities and business and economic constituencies throughout the state and nation.

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COST-EFFECTIVE COLLABORATION BETWEEN THE UNITED STATES AND PERUVIAN NAVIES AND A PERUVIAN UNIVERSITY TO PROVIDE IMPROVED PUBLIC HEALTH MEASURES AGAINST DENGUE AND YELLOW FEVER IN PERU

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In Peru, there are no formal medical entomology programs available at the graduate or post-graduate, and very limited training at technical levels. However, Peru is endemic to many medically-important insects, including *Aedes aegypti*, which vectors dengue and yellow fever virus pathogens into human populations. An international collaboration was formed between the Peruvian Instituto de Medicina Tropical "Daniel A. Carrion" of San Marcos University (IMT DAC UNMSM), the Entomology Department of the United States Naval Medical Reserach Unit No. 6 (NAMRU-6), and the Sistema de Alerta DISAMAR of the Peruvian Navy Clinic. This collaboration resulted in the provision of formal medical entomology training specifically focused upon surveillance and control of *Aedes aegypti*, the mosquito vector of dengue and yellow fever viruses, to Peruvian naval Nurses, who will be stationed in remote locations throughout Peru during their Naval careers. This collaboration has been organized as a long-term collaboration, with the goal of providing this training 2-3 times each year to new active-duty Peruvian nurses prior to their deployment to remote areas in Peru that are endemic to these debilitating diseases.

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CHANGES IN RELATIVE ABUNDANCE OF *ANOPHELES GAMBIAE* S.S. AND *AN. ARABIENSIS* IN SUBA DISTRICT, WESTERN KENYA: ITS RELATION TO BED NET COVERAGE

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Coverage of insecticide treated bed net has increased considerably in Kenya for the past few years. Since insecticide treated nets kill indoor mosquitoes, the relative abundance of *Anopheles gambiae* s.s. to *An. arabiensis* may decrease, because *An. gambiae* is more endophilic and anthropophilic. We compared the current relative abundance of both species with that in the past in Suba District. Then, we examined the relationships between relative abundance and bed net coverage. Anopheline larvae were collected from the same areas in 2009 and 2010 that were surveyed by a study in 1998. Indoor resting anophelines were also collected in the same villages in 1999 and 2008. Moreover, we monitored the relative abundance and bed net coverage periodically from 2007 for three years. In the larval survey, over 90% of collected larvae were *An. arabiensis* in 2009 and 2010 while approximately 70% were this species in 1998. The density of indoor resting anophelines in 2008 was one seventh of that in 1998. The decrease was mainly due to the decrease of *An. gambiae* s.s., which increased the relative abundance of *An. arabiensis* from 9.3% to 39.2%. The three-year survey revealed non-linear relationships between bed net coverage and relative abundance of *An. arabiensis*. When coverage exceeded 0.7 nets per person, the density of *An. gambiae* s.s. decreased, and the relative abundance of *An. arabiensis* increased. However, the trend was unclear below 0.7 nets per person. The results support the notion that bed net coverage alters the relative abundance of malaria vector species. In an area where *An. arabiensis* is dominant, the effectiveness of bed nets may be hampered.

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LA CROSSE ENCEPHALITIS IN EASTERN TENNESSEE: EVIDENCE OF INVASIVE MOSQUITO (*AEDES ALBOPICTUS* AND *OCHLEROTATUS JAPONICUS*) INVOLVEMENT IN THE TRANSMISSION OF AN INDIGENOUS DISEASE

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La Crosse encephalitis virus (LACV), family Bunyaviridae, is an important cause of pediatric encephalitis in the United States. The virus is transmitted by the bite of infectious mosquitoes, primarily the native tree- hole mosquito *Ochlerotatus triseriatus*. Since being characterized in the 1960s, human cases have been concentrated in the upper- Midwestern states where the virus is considered endemic. Approximately 80- 100 cases are reported annually. While death is rare, symptoms can be severe and often require hospitalization. In the mid 1990s, a new focus of the disease was recognized in West Virginia, North Carolina and eastern Tennessee. One hypothesis for the establishment of this new focus is that the invasive mosquito, *Aedes albopictus*, may be acting as a novel vector in this area. A third mosquito species, *Oc. japonicus*, has recently become established in the region and is also a competent vector of LAC in the laboratory. The potential for invasive mosquitoes to modify disease epidemiology is large. These three species occupy many of the same larval habitats and the invasive species may have an effect on the local mosquito community due to resource competition. To test the invasive vector hypothesis, mosquito eggs, larvae, and adults were collected weekly from six recent human case sites in eastern Tennessee from May - August 2010. Three pools of *Ae. albopictus*, one pool of *Oc. japonicus* and eight pools of *Oc. triseriatus* were LACV positive by PCR. Additionally, eleven of the twelve positive pools came from mosquitoes collected as eggs, indicating active transovarial transmission. This is the second study to find field caught mosquitoes positive for LACV in Tennessee with the first sample being *Ae. albopictus* from 1999. To our knowledge, this is the first recorded report of *Oc. japonicus* being naturally infected with LACV and in close association with human habitation. This study provides further evidence that invasive species may have changed the epidemiology of a vector-borne disease in the United States. Viral assays are ongoing.

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INVESTIGATIONS INTO MOSQUITO BLOOD FEEDING PATTERNS ON WILDLIFE AND A POTENTIAL ROLE FOR BATS IN ARBOVIRUS TRANSMISSION CYCLES IN UGANDA

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Zoonotic and vector-borne pathogens have comprised a significant component of emerging human diseases in the last decade. Uganda has a history of enzootic and epizootic arbovirus activity and has been predicted as a hot spot for disease emergence. Serological evidence

exists documenting exposure of various East African bat species to many arboviruses including Rift Valley fever, Yellow fever, West Nile, Usutu, Sindbis, Bunyamwera, and Zika viruses, however the role of bats in arbovirus transmission cycles is poorly understood. While collecting mosquitoes as part of an emerging arbovirus surveillance project in Uganda, we obtained blood-engorged *Culex* mosquitoes which had fed on fruit bats in both Semliki and Maramagambo Forests. To follow up on these observations and investigate the role of bats in arbovirus transmission cycles, blood samples from *Rousettus aegypticus* bats collected from the python cave in Maramagambo Forest were screened for West Nile, Yellow Fever, Dengue, Chikungunya, and O'nyong'nyong viruses by plaque reduction neutralization test (PRNT), and mosquitoes were trapped from around the vicinity of the cave. Blood and tissue samples were also collected from various fruit and insectivorous bat species in Kampala, Uganda and tested for evidence of arbovirus infection by PRNT and virus isolation. Serological and virological evidence will be presented on the arbovirus exposure history of several species of bats in Uganda. The blood feeding patterns of mosquitoes on a diversity of wildlife species in Uganda and potential enzootic arbovirus transmission cycles between mosquitoes and wild vertebrates including bats will be discussed.

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LANDSCAPE ECOLOGY OF DENGUE AND CHIKUNGUNYA SYLVATIC VECTORS IN SOUTHEASTERN SENEGAL

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Dengue (DENV) and chikungunya viruses (CHIKV) circulate in a sylvatic transmission cycle between non-human primates and arboreal Aedes spp. in Kedougou, Senegal, and several studies have shown a low incidence of infection by both sylvatic viruses in humans in West Africa as well. Although humans are probably infected by sylvatic vectors, the extent and mechanisms of contact between humans and sylvatic vectors remains unknown. To gain insight into the role of different mosquito species in both enzootic transmission in primates as well as spillover into humans, between 2009 and 2010 we monitored the distribution of a broad array of mosquito species in five landscape classes (forests, savannahs, barren, agricultural, and villages) in the Kedougou area. Mosquito were collected monthly in each of the landscape classes from 18:00 to 21:00 hrs and identified to species. Among 39,799 mosquitoes collected, the most and least abundant species were *Ae. vittatus* and *Ae. aegypti*, respectively. The abundance of *Ae. vittatus, Ae. luteocephalus* and *Ae. aegypti* peaked in June, while that of other species peaked twice between July and November, 2009. The preferred habitat of *Ae. africanus, Ae. luteocephalus* and *Ae. taylori* was the forest canopy, while the others species were distributed more evenly across the five landscape classes. CHIKV was detected by real-time PCR assay and/or virus isolation in 39 pools of mosquitoes, including previously recognized (*Ae. furcifer, Ae. taylori, Ae. dalzieli, Ae. luteocephalus, Ae. africanus, Ae. aegypti, Ae. neoafricanus*, *Ae. hirsutus, An. funestus, An. coustani*, *Ma. uniformis*) and potentially new (*Ae. metallicus, Ae. centropunctatus, Ae. hirsutus, An. domicola* and *Cx. poicilipes*) CHIKV vectors. Infection rates showed temporal and spatial variation. No DENV was detected. Our findings provide insight to the ecology of sylvatic vectors of DENV and CHIKV in a changing environment affected by urbanization and deforestation associated in part with mineral exploitation.

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EVOLUTIONARY HISTORY OF *AEDES AEGYPTI*: A GLOBAL PERSPECTIVE

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Aedes aegypti is the principal vector of both dengue and yellow fever viruses worldwide. A human commensal, this mosquito species has successfully invaded much of the tropical and subtropical world over the past few centuries. Though *Ae. aegypti* is often treated as a homogenous species, populations of the mosquito differ markedly in their association with human habitats, as well as in their ability to transmit dengue viruses. Recent microsatellite work in our lab suggested that the African sylvan subspecies, *Ae. ae. formosus*, is ancestral to the worldwide domestic form (*Ae. ae. aegypti*), but that close human association has likely evolved multiple times independently in *Ae. aegypti*. In order to more formally test hypotheses of ancestry and trait evolution, we sequenced 4 variable nuclear loci from 167 individuals representing 17 global populations of *Ae. aegypti*. The same regions were sequenced in two closely related species to provide outgroups for rooted phylogenies. In addition, a sequenced RAD (restriction-site associated DNA) approach was undertaken to explore at a fine-scale the history and colonization of *Ae. aegypti* out of Africa and across the global tropics and subtropics. This method allows simultaneous detection and screening of thousands of SNPs across the *Ae. aegypti* genome. Bar-coded RAD libraries were successfully constructed from 136 individual mosquitoes (8 each from 17 populations) and sequenced on an Illumina platform. Both the 4 sequenced nuclear loci and the RAD markers confirm African *Ae. ae. formosus* as the ancestral form of the species, and support multiple "domestication" events. However, the RAD markers are significantly more sensitive at detecting population structure and tracing the invasion history of this important vector arthropod out of Africa and across the world. In addition, the SNPs detected in our RAD analyses will prove useful in future association mapping studies, such as those for important epidemiological traits including vector competence for dengue and human host preference.

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HOST ATTRACTION OF ANOPHELINES IN SOUTH HALMAHERA, INDONESIA

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The feeding behaviors of Indonesian malaria vectors remain largely uncharacterized. A Latin square design was used to compare anophelines attracted to human, cow, and goat-baited tents. The experiment was carried out for 12 nights in August 2010 in Saketa village in South Halmahera, Indonesia. Specimens were collected from the inside walls of baited tents every hour from 18:00 to 7:00 hours and were morphologically identified. A subset of bloodfed specimens were analyzed using a bloodmeal diagnostic PCR assay. 1,235 *Anopheles* specimens of nine different morphological species were collected over 12 catch nights. These morphological species included *An. farauti, An. hackeri, An. indefinitus, An. kochi, An. punctulatus, An. subpictus, An. tessellatus, An. vagus*, and *An. vanus*, all of which have been previously shown to be capable of transmitting *Plasmodium* parasites. 1024, 137, and 74 anophelines were collected in cow, goat, and human-baited tents, respectively. Bloodmeal analysis of specimens collected in the human-baited tent indicate a low level of multiple host blood feeding. Morphological species distribution was similar between the cow and goat-baited tents, with a majority (44% and 36%) of *An. indefinitus*,

but different for the human-baited tent, with a majority (41%) of *An. vagus*. Eight of the nine morphological species represented in this study were captured on each of the three hosts, suggesting a plasticity in host attraction behavior. Multiple host feeding and flexibility in feeding behavior could have important implications for malaria control.

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ASPECTS OF ECOLOGY OF POTENTIAL RIFT VALLEY FEVER VIRUS MOSQUITO VECTORS, KHARTOUM STATE, SUDAN

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Rift valley fever epidemics are disruptive and expensive to local and regional economies. After a devastating outbreak of Rift Valley Fever in khartoum state, Sudan 2007; ecological baseline surveys were conducted in Khartoum State, Sudan, during the rainy season (end of July to the beginning of September) 2008 in order to identify mosquito species present and evaluate their emergence and survivorship. Larval identification of species of Culicine and Anopheline mosquitoes present in Khartoum State taken from five study sites represents Khartoum state indicated that *Anopheles arabiensis* is the only species of the Anopheline mosquitoes found. Three species of culicine mosquitoes were found: *Culex quinquefasciatus*, *Cx univittatus* and *Cx arbeeni*, Species of *Aedes* were found in irrigated schemes at one study site and was absent from the other four study sites, these species were *Ae. vittatus* and *Ae. vexans*, whose presence was recorded after the onset of the rainy season.the same breeding site was first occupied by *Ae.vittatus* then *Ae. vexans,* with an interval of habitat drying. Daily emergent adults Culicine and Anopheline mosquitoes present were taken from randomly selected breeding sites in the five study sites, population measurements were performed. The absolute number of emergent adults was obtained by collecting mosquitoes under net-traps covering the breeding sites. Records were taken each day for seven constitutive days, synchronized emergence of males and females was observed at all the study sites, showing an overall marked predominance of females in emergence trap catches. Adult survival rate was the most important factor determining the stability of the population and total egg production. Females that become infected when taking a blood meal must survive throughout the incubation period of the pathogen. Under controlled laboratory environment, effect of food types (sucrose 10%, sucrose 10% and blood diet) on longevity of adult female mosquitoes was conducted, sugar-fed and blood-fed mosquitoes exhibited very high percentage of surviving rates beyond the 15 days (incubation period for RVFV),However these have varied among the five study areas. Also results indicated prolonged survival of sugar-fed female mosquitoes more than blood and sugar fed females, this served to increase survivorship of females until they find the appropriate host.

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MAIN MOSQUITO BREEDING SITES FOR *AEDES AEGYPTI* IN THE PAN-AMERICAN HIGHWAY: CUCUTA-PAMPLONA AREA (NORTE DE SANTANDER - COLOMBIA) IN 2010

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Aedes aegypti is the principal dengue vector in Colombia where dengue transmission is limited by the presence of the vector; unfortunately in this country, the presence of *Ae. aegypti* has been documented up to 2200 m.a.s.l. Norte de Santander is the second most endemic area for dengue in the country. Previous studies have associated travel and transport as key factors in the spread of diseases and vectors. With this pilot study, we investigated the main breeding sites and mosquito larva species on the highway from Cucuta (325 m.a.s.l) to Pamplona (2342 m.a.s.l) in 75km distance. We found that tires where the main breeding site followed by plastic containers and small pools along the way. The main specie collected was *Ae. aegypti* followed by *Culex quinquefasciatus*. *Anopheles* mosquitoes were not found in the highway area. Tire repair shops were the places with the highest number of infected tires; we also found abandoned tires infected with mosquito larva.

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A COMPARATIVE EVALUATION OF SIX DIFFERENT MALARIA VECTOR COLLECTION METHODS IN LOW-LYING MALARIA ENDEMIC REGIONS OF WESTERN KENYA

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Outdoor biting and other forms of behavioral adaptation by malaria vectors to domestic insecticide-based control measures may compromise the sensitivity of conventional sampling tools operating indoors such as light traps or indoor resting catches, thus preventing effective surveillance and management of vector populations. We evaluated six different vector collection methods to optimize a protocol for operational sampling of malaria vectors robust to variations in vector behavior, notably variations associated with the presence of important malaria control methods. Over 30 days, we replicated a Latin square design 10 times at sites in 4 districts in western Kenya: Kisumu, Bondo, Nyando and Rachuonyo. Each site consisted of 3 locally representative houses through which the six different sets of trapping methods were rotated every 3 nights in a random order of three possible arrangements: 1) Indoor human landing catch (HLC) and outdoor HLC, 2) CDC Light trap placed beside an occupied insecticide-treated net indoors combined with Ifakara tent traps outdoors, and 3) Window traps to catch exiting mosquitoes combined with both pot and box formats of resting traps placed both indoors and outdoors. At each site, a fourth house was selected for pyrethrum spray catch (PSC). The top collection methods with their corresponding number of *Anopheles* per collection effort were PSC (10.5), HLC indoor (3.0), Light trap (3.0) HLC Outdoor (2.8) and Ifakara tent traps (2.7). Resting Boxes and Pots positioned both indoors and outdoors caught less than 1 *Anopheles* per collection effort. HLC outdoor collected the highest amount of *Culex* at 77.4 per collection effort. Irrespective of the intensity or type of insecticide based vector control method in place and of biting behavior of the local malaria vectors, we conclude that pyrethrum spray catch is the most sensitive method for vector collection in low lying malaria endemic regions of western Kenya.

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HETEROGENEOUS FEEDING PATTERNS OF *AEDES AEGYPTI* IN HOUSEHOLDS IN IQUITOS, PERU

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Heterogeneous biting by female mosquitoes can significantly alter transmission of mosquito-borne pathogens. Previous studies show *Aedes aegypti*, the primary vector of dengue viruses (DENVs), more frequently bite individuals with higher body mass index (BMI). Because BMI increases with age, we expect positive linear relationship with age and biting. Studies show, however, that young adults receive more bites than older adults. Factors such as sex, mosquito exposure time and previous DENV infection should, therefore, be used to analyze heterogeneous feeding patterns. Between October 2009 and November 2010, 2,035 interviews with 280 participants were conducted in 19 households in Iquitos, Peru.