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QUANTITATIVE PCR-BASED ASSESSMENT OF *ANGIOSTRONGYLUS CANTONENSIS* IN *RATTUS RATTUS* FROM HAWAII

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Angiostrongylus cantonensis is the most common parasite causing human eosinophilic meningitis worldwide. This nematode's infectious larvae develop in mollusks, but can also be present in paratenic hosts. The major route of infection is thought to be accidental consumption of infected mollusks in fresh produce or other foods. A survey performed in 2007 on mollusks collected in Hawaii main island showed an infective rate ranging from 24 to 78% depending on the mollusk species analyzed. The severe cases of eosinophilic meningitis recently reported could be due to overexposure of humans to highly infected mollusks; there are no measures in place to control the spread of *A. cantonensis* in Hawaii. The aim of this study was to determine the prevalence of *A. cantonensis* in rats, the most efficient definitive hosts for this parasite. A total of 62 rats were trapped in 4 Hawaiian communities. The presence of *A. cantonensis* was initially assessed by morphologic identification of adult worms in lungs and hearts of the animals. DNA was then extracted from 28 randomly selected lung and heart tissue samples obtained from these animals. The DNA extracts were evaluated using a quantitative TaqMan PCR validated for diagnostic detection of *A. cantonensis*. A total of 60 % (n=37) of the rats were positive by morphology, while 100% of the tissue samples examined with the PCR were positive. A quantitative analysis of the PCR results indicated that 32 % (n=9) of the rat samples contained *A. cantonensis* DNA corresponding to more than 100 larvae in approximately 25 mg of tissue. Together with previous studies, this data indicates that angiostrongyliasis may be a more serious public health issue in Hawaii than currently estimated and that measures to control its spread in mollusks and rodents may be warranted.

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INCREASED BASOPHIL RESPONSIVENESS AFTER ANTHELMINTIC TREATMENT IN A COASTAL ECUADORAN POPULATION

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As basophils function as allergy effector cells and may be involved in initiating and perpetuating allergic diseases, it is possible that much of the allergy-protective effect conferred by helminths is through their effect on basophils. Recently, we demonstrated that basophil responsiveness decreases in murine models of chronic helminth infection. In this study, we tested the hypothesis that basophil responsiveness is suppressed during helminth infection of humans. Twenty-three children infected with intestinal helminths were identified by parasitological examination of stool samples in Esmeraldas, Ecuador. Peripheral blood cells from these children were washed twice and stimulated with increasing concentrations of anti-IgE and 5 µg/ml ionomycin. Basophil activation was determined by measuring histamine release into the supernatant using a competitive ELISA. Children were then treated with ivermectin and 2 doses of albendazole and histamine release from blood basophils measured again two weeks later. A two week timepoint was chosen as basophil half-life is estimated to be between 5-8 days. Significantly more histamine was released from basophils after anthelmintic treatment when blood was stimulated with 0.5, 0.125, and 0.031 µg/ml anti-IgE (mean % of total histamine release from infected children before treatment = 40.38, 43.12, 31.49 vs. 55.17, 60.53, 44.89 from children post-treatment, $p < 0.01$, $p < 0.001$, $p < 0.05$). A similar increase in histamine release was seen when stimulating with ionomycin (mean % of total histamine release from infected children before treatment = 49.30 vs. 76.71 from children post-treatment, $p < 0.0008$). These results suggest that the ability of basophils to respond to IgE-dependent and IgE-independent activation is suppressed during intestinal helminth infection of humans. This suppression appears to require the continuous presence of helminths as basophil histamine releasability increases shortly after helminths are eliminated. These findings may explain why helminth infections protect against allergic disease.

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SPATIAL PATTERNING OF HEALTH DISPARITIES AND ENVIRONMENTAL FACTORS ASSOCIATED WITH *ASCARIS LUMBRICOIDES* PREVALENCE IN BOLIVIA

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Infection with *Ascaris lumbricoides*, a soil-transmitted helminth (STH), affects as many as one billion people worldwide, and causes an estimated 10.5 million disability-adjusted life-years (DALYs) lost. STH prevalence data for Bolivia is sparse. A 2009 literature review found only 7 studies in the last 25 years that offer prevalence estimates for any region of Bolivia. A review conducted in 2006, that reported data from 1976-2003, showed that *A. lumbricoides* infection prevalence varies geographically across Bolivia, from 1-28% in the Western high plains to 15-96% in the Eastern tropical lowlands. Although data on STH prevalence in Bolivia is patchy, there is information available on environmental and social risk factors known to be associated with *A. lumbricoides* prevalence. Environmental conditions, particularly temperature and soil moisture, largely determine global distribution of *A. lumbricoides*. Social factors, such as piped water, sanitation and improved housing (absence of dirt floors), are associated with lower STH infection. Using spatial autocorrelation statistics (Moran's I and Local Indicator of Spatial Association [LISA]), we examined the spatial distribution of risk factors associated with increased prevalence of *A. lumbricoides* infection. Subsequently, we created a risk model integrating environmental and social factors with previous community-level *A. lumbricoides* survey data. This model is a cost-effective way to predict areas of high and low STH endemicity in the absence of nationally representative prevalence data. The model can be used to inform efforts to fill information gaps on prevalence and intensity of STH infections and better focus mass drug administration campaigns and water, sanitation and housing infrastructure investments to mitigate STH infections across Bolivia.

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HOOKWORM AND *TRICHURIS* INFECTIONS ASSOCIATED WITH ANEMIA DURING PREGNANCY

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The objective of this study was to assess changes during pregnancy in the association between the intensity of soil-transmitted helminth (STH) infections and hemoglobin (Hb) levels, following iron supplementation and mebendazole administration, in an endemic region of Peru. Data from a randomized clinical trial on the effect of mebendazole on birthweight were re-analyzed. Pregnant women (N=935) recruited in their second trimester provided baseline data on STH infections and anemia. All women were given iron supplements (60 mg/d); half were randomly allocated to receive single dose 500mg mebendazole and half placebo. Hb was also determined in the third trimester. Mean Hb level increased from 11.03 to 11.39 g/dL between the second and the third trimester. Hookworm

(prevalence 46.3%) and *Trichuris* (76.1%) infections, but not *Ascaris* (63.9%), in the second trimester were independently associated with lower Hb in both the second and third trimester. *Trichuris* >1200 epg was also associated with a lower increase of Hb between the second and the third trimester (ΔHb = -0.26 g/dL, p=0.026). The prevalence of anemia (Hb < 11 g/dL) decreased from 46.8% to 34.7% between the second and the third trimesters. Hookworm and *Trichuris* infections were significantly and independently associated with increased risk of anemia in the third trimester. The adjusted OR for hookworms >600 epg was 1.52 (95% CI 1.01 to 2.28), and the adjusted OR for *Trichuris* >1200 epg was 1.72 (95% CI 1.14 to 2.61). Mebendazole treatment was not associated with Hb or with the prevalence of anemia, and did not modify the relationship between helminths and Hb. In conclusion, in pregnancy, both hookworm and *Trichuris* infections are associated with increased risk of anemia. Infected women remain at higher risk of anemia throughout pregnancy, irrespective of mebendazole administration. These data suggest that more effective deworming interventions be targeted to pregnant women in endemic areas.

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TUBERCULOSIS (TB) CONTROL AMONG HEALTH CARE WORKERS (HCW), RESEARCHERS, TRAINEES AND TRAVELERS TO A TB-ENDEMIC AREA WITH AN ACADEMIC, INTERNATIONAL, MEDICAL EXCHANGE PROGRAM

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Although individuals from low TB burden countries experience an increased risk of TB infection when traveling to high burden countries for medical training or service, degree of risk has not been well quantified. Improved knowledge will aid development of guidelines for TB screening, pre/post-travel education and risk reduction. 608 individuals who traveled to Eldoret, Kenya with the AMPATH medical exchange program between July, 2004 - June, 2009 were invited to complete an online survey. Survey questions included demographic characteristics, pre-travel TB counseling, in-country activities, and post-travel TB testing. 418/608 (69%) responded. The majority were young adults (65% between 22-40 years old) and 55% were female. 7% had chronic medical conditions predisposing to increased risk of TB. Pre-travel, respondents sought travel advice from a number of different sources: travel clinic (54%), CDC travel website (31%). 58% reported that TB prevention was discussed in travel preparations. Pre-travel, 81% reported negative Tuberculin skin test (TST) or Interferon Gamma Release Assay (IGRA); 11% reported unknown infection status. Most commonly reported duration of visits was 5-8 weeks. Most-common activities included direct medical care in adult medical ward (49%) and non-direct medical care activities in a lab or on hospital grounds (38%). Respondents also commonly visited Kenyan homes (65%) and used public transport (40%). 13 individuals (3 under age 21) converted to a positive TST during their travel. One reported an episode of active TB. 113 (28%) of survey participants reported "ideal" care (definition: pre-travel TST (within one year of travel), pre-travel counseling, and a repeat post-travel TST). In conclusion, travelers to TB endemic areas with international, medical exchange programs are at significant risk for TB infection. Many receive inadequate pre and post-travel TB counseling and testing. Efforts should be made to improve TB education for program participants. Further study is needed to quantify the risk of TB infection.

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MOLECULAR EPIDEMIOLOGY OF BOVINE TUBERCULOSIS ACROSS THE UNITED STATES-MEXICO BORDER: SUPPORTING STRATEGIES FOR ANIMAL AND PUBLIC HEALTH INTERVENTIONS

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Bovine tuberculosis, caused by Mycobacterium bovis, is a major global cause of respiratory disease and decreased production in beef and dairy cattle. Human infections with M. bovis represent an emerging public health concern in immigrant communities in the US. The epidemiology of zoonotic M. bovis infection is complex, but may be associated with exposure to contaminated animal products such as soft cheeses and unpasteurized milk. Elucidating the epidemiology of M. bovis prevalence and transmission among cattle in the US, particularly among animals imported from Mexico, can be an important component of strategies to reduce the economic and public health impacts of this disease. Using techniques to genotype M. bovis strains, such as spoligotyping and variable number tandem repeat (VNTR) analyses, we have examined the molecular epidemiology of bovine tuberculosis outbreaks in southwestern states in the US. In the majority of these outbreaks, the data indicate that the M. bovis isolates do not display the genotypic profiles common to isolates present in cervids, which are considered to be a major source of bovine TB infections among cattle in the central and eastern regions of the US. Rather, the genotypic profiles for the M. bovis isolates recovered from US animals are representative of those documented to occur in cattle originating from Mexico. Efforts are underway to expand databases of molecular fingerprinting data for M. bovis isolates obtained from cattle and wild animals to enhance the USDA's abilities to identify foci of the disease in cattle populations, and improve efforts to control bovine tuberculosis within the US.

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QUALITATIVE FINDINGS AND IMPLICATIONS FOR SCALING UP AN IMPROVED COOKSTOVE PROJECT IN RURAL KENYA

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Traditional indoor, three-stone cooking fire pits, a staple in rural households in resource-poor countries are not without costs to human health and the environment. We conducted a pilot intervention to promote the purchase and use of an improved cookstove in rural Kenya. In order to improve the scaling up of the stove promotion effort to the larger community, we conducted a qualitative inquiry to better understand the activities and strategies vendors used to promote the stove, the motivations of community members to purchase and use the stove, and the perceived benefits and challenges of stove use among very poor Luo women. Purposive sampling was used to recruit 10 health promoter-stove vendors and 30 stove purchasers of the stove in the Luo community. Formative research was conducted through qualitative semi-structured key informant interviews with a topic guide. Data were transcribed and imported into Atlas-ti and a thematic analysis conducted to identify patterns representing a comprehensive picture of personal and collective experiences. Women who purchased the improved cook stove reported the most influential promotion strategies were interpersonal communication through informal social networks, observations of someone using the stove, and actual cooking demonstrations. Luo women reported the need for less firewood, fuel costs savings, reduced smoke, increased cooking efficiency, and reduced eye irritation, lung congestion

and coughing as major benefits. While there were notable financial and health benefits along with a decreased workload for women, the cost of the stove and the associated role of men in decision making for household purchases appeared to be possible barriers to wider spread adoption. In conclusion, qualitative findings noted that the price of the stove will need to be reduced or subsidized if there is a commitment for community-wide access to the health benefits of this newer technology. Promotion activities employing interpersonal communication and cooking demonstrations were critical for scaling up the project.

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UNDERSTANDING RESPIRATORY INFECTION IN BANGLADESH: COMMUNITY PERCEPTIONS, SOCIAL NORMS AND CURRENT PRACTICES

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Respiratory infections are the leading cause of childhood death in Bangladesh. Non-pharmaceutical interventions such as respiratory hygiene (covering the nose and mouth when coughing or sneezing and washing hands after having contact with respiratory secretions and contaminated objects) may be effective in reducing infection transmission. This formative study explored community perceptions about respiratory infections, why they occur, how they are spread, and the preventive measures that can be taken to protect themselves and their families. We used semi-structured interviews and focus group discussions with community members, leaders and school children to explore respiratory infection and hygiene related perceptions, social norms and practices. From both the semi-structured interviews and focus group discussions, we found that our informants were not familiar with the term "respiratory disease" as translated into Bengali. When asked to give examples of respiratory diseases, they named diseases some of which had no relation to respiratory function. The informants identified a number of social norms related to respiratory hygiene, including covering coughs or sneezes, turning face away during sneezing and coughing, and not spitting on the ground, but very few people practice these. During semi-structured interviews, all informants cited hot/cold weather changes and using cold water as the cause for catching cold during the previous twelve months. Yet, when asked about modes of transmission, these were associated with close contact with the breath, spit or cough droplets of a sick person. The most effective way to prevent respiratory infection was to avoid these. Although most of our informants perceived that handwashing after coughing and/or sneezing might prevent illness, most of them felt that this was not practical after every event. In conclusion, informants related their personal experience of catching a cold to the local explanatory model of hot-cold imbalance including perceived changes in body temperature and environment. But for transmission and prevention they related to both the biomedical construct and the cultural contagion model. This gap provides useful insights for message development that can be incorporated into "culturally compelling" communication interventions to make people conscious about respiratory hygiene and to increase their self efficacy.

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INCIDENCE OF INFLUENZA INFECTION IN EARLY INFANCY IN SOUTH ASIA

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Young infants 0-6 months of age in the US have a reported influenza incidence of 12% and high rates of 'flu hospitalization. Historically influenza has not been considered a frequent or serious illness of infants in the tropics, partly because of the lack of data. We report the serology and 'flu antigen data from 131 infants of mothers who received pneumococcal vaccine as controls in a 'flu vaccine study. A full description of the Mother'sGift trial has been published. 340 pregnant women in Dhaka were randomized to receive pneumococcal or trivalent inactivated influenza vaccine. From Aug 2004 - Oct 2005, non-'flu vaccine control mothers and their infants were visited weekly from birth to 6 months to record symptoms of febrile respiratory illness. Influenza antigen was tested by rapid antigen-detection test (RADT) in symptomatic infants. A standard hemagglutination inhibition (HAI) assay was done on infant sera collected at birth, 10, and ~ 22 weeks of age. We defined serological infection as a $\geq$ 4-fold increase of the expected declining later titers compared to the earlier titer. Of 131 infants with prospective data 29 experienced 31 serologically-defined influenza infections. Adding 10 distinct RADT-proven infections resulted in a total of 41 influenza infections or a cumulative incidence of 31.3/100 0-6 month old infants (95% CI: 23.6-41.4). Maternal HAI data is being analyzed. In conclusion, additional studies of the incidence of influenza in young infants in Asian and other tropical regions should be carried out to define the burden of preventable influenza illness. Antenatal immunization is a proven prevention strategy in this vulnerable group.

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PREVENTIVE MEASURES ASSOCIATED WITH PROBABLE CASES OF A (H1N1) INFLUENZA IN INHABITANTS OF THE FEDERAL DISTRICT OF MEXICO CITY

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When pandemic A (H1N1) Influenza (flu) broke out in the spring of 2009 in Mexico, the Ministry of Health established and profusely promoted preventive and control measures that could slow or stop transmission. The objective of this study was to evaluate if inhabitants of the Federal District of Mexico City understood and implemented the measures recommended and if there was an association with probable cases of the flu. An ad-hoc questionnaire was given to 4003 subjects who voluntarily agreed to participate in a household survey aimed at detecting cases of influenza between August and September 2009. The average age was 46±16 years of age and 64% were women. Most people reported to wash hands and open windows at home, but only 32% covered their nose with the forearm when sneezing, 34% used alcohol gel and 70% kept shaking hands or kissing when greeting others. The frequency of symptoms of flu like disease occurring from January to September 2009, were: 29% running nose, 25% coughing, 17% head, muscle or joint ache and 7% sudden high fever and 11% had 3 or 4 of these symptoms (probable cases); 62% attended a physician when flu symptoms appeared, 22% self-medicated and 17% did not do anything. Significant associations were found between probable cases and not washing hands, and also for

inadequate techniques when sneezing or greeting. The potential impact of these factors on the frequency of probable cases, calculated as the attributable risk for the exposed, ranged from 29 to 55%. The fact that a third of the population used the appropriate technique to cover their nose while sneezing or used alcohol gel, measures newly recommended by the government, suggests that people follow novel advices, while a similar proportion changed inadequate habits, such as touching or kissing. This study was performed only 3 months after the pandemic started and allowed identifying public health information that should be intensified or modified, especially because the survey was carried out among the general population and not only in diagnosed cases.

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OUTBREAK OF PANDEMIC INFLUENZA A (H1N1) IN RWANDA, OCTOBER 2009

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On October 9th, 2009 Pandemic Influenza A (H1N1) outbreak was confirmed in Rwanda. The index case a Rwandan female, 39 years old traveled to US and arrived in Kigali on October 3rd, 2009. She presented with fever, cough and joint pains on October 4th, 2009. We describe the epidemiology of this novel virus in Rwanda. Methods: We undertook epidemiologic investigations around the index case. We collected respiratory specimens from suspect cases using a WHO case definition and tested specimens by real time RT-PCR at the National Reference Laboratory. From October 8th through October 31st, 2009, we tested 810 specimens. Of these, 127 (15.7%) were positive for pandemic H1N1. Of confirmed cases, 81 (63.8%) were children <15 including 17 children < 5 years old, 74 (58.2%) were female, 35 (27.6%) were in a high-risk category. Two (1.6%) cases were hospitalized and subsequently discharged; all other cases were outpatients. One hundred and seventeen (98%) confirmed cases received treatment with oseltamivir and two received post-exposure prophylaxis. Confirmed cases occurred in two referral hospitals, five schools all in Kigali city and in 46 households in Kigali City, northern and western Provinces. The attack rates at the two schools with the largest H1N1 clusters were 2.4% and 3.8%. Twelve (9.4%) confirmed cases of third-generation transmission occurred and 87 (68.5%) cases had unknown exposure to known confirmed cases. In conclusion, transmission of Pandemic influenza A (H1N1) was confirmed in Rwanda and documented in diverse settings, including health facilities, schools, and households. Most confirmed cases were female and children. Disease appears mild, similar to that seen in other East African countries. We recommended mitigation measures, including public education on home care, enhanced training of health care workers, use of targeted laboratory testing and treatment, and robust surveillance.

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SPATIAL AND TEMPORAL VARIATION IN VECTOR COMPETENCE OF *CULEX PIPIENS* AND *CX. RESTUANS* MOSQUITOES FOR WEST NILE VIRUS

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Vector competence, the probability that a vector will transmit a pathogen after feeding on an infected host, is known to vary among vector species, populations, days since feeding, and temperature during the extrinsic incubation period. However, the extent of spatio-temporal variability and consistency in vector competence of populations is not known. We examined vector competence of *Culex* pipiens Linnaeus and *Cx. restuans* Theobald mosquitoes for West Nile virus (WNV) collected over three years from sixteen sites to measure spatial and temporal scales of variation in vector competence. We found extreme variation with 0-52% of mosquitoes transmitting WNV at a single site between different sampling periods, and similar variation across populations. However, we also found that within a smaller geographic range vector competence varied somewhat synchronously, suggesting that environmental and population genetic factors might influence vector competence. These results highlight the spatio-temporal variability in vector competence and the role of local processes.

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WEST NILE VIRUS ENVELOPE PROTEIN GLYCOSYLATION AFFECTS VIRUS-VECTOR INTERACTIONS IN A SPECIES-SPECIFIC MANNER

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Many, but not all, strains of *West Nile virus* (WNV) contain a single N-linked glycosylation site on their envelope (E) proteins. Previously, we found that a WNV that lacked the glycosylation site (ENONGLY) replicated less efficiently than glycosylated WNV (EGLY) in *Culex pipiens* and *Cx. tarsalis* mosquitoes. Additionally, transmission of WNV by mosquitoes that fed on ENONGLY was associated with viral reversion to a glycosylated phenotype. We have recently expanded these studies to include an additional important WNV vector, *Cx. quinquefasciatus*. To determine if replicative differences were present in this species, we inoculated mosquitoes intrathoracically with 10pfu of either ENONGLY or EGLY and determined titers in the mosquitoes from 1 to 10 days post inoculation. As we previously observed with *Cx. pipiens* and *Cx. tarsalis*, replication of ENONGLY was decreased relative to that of EGLY in *Cx. quinquefasciatus*. Similar decreases in replication were observed in individual mosquito tissues (midguts and salivary glands). To determine whether viral genotype affected infectivity and transmission in *Cx. quinquefasciatus*, we fed mosquitoes a bloodmeal containing either EGLY or ENONGLY and examined infection, dissemination, and transmission rates at various days post-feeding (dpf). At all dpf, infection rates were greater in mosquitoes that had fed on a bloodmeal containing EGLY, similar to previous results in *Cx. pipiens*. However, dissemination and transmission rates were higher at 7 and 14 dpf in mosquitoes that fed on a bloodmeal containing the ENONGLY virus. In contrast to previous results with both *Cx. pipiens* and *Cx. tarsalis*, none of the viruses transmitted by *Cx. quinquefasciatus* following feeding on the ENONGLY bloodmeal exhibited reversion to EGLY phenotype. Taken together, these data suggest that E protein glycosylation

affects WNV-vector interactions differently in different *Culex* mosquito species and confirms earlier studies with WN02 and NY99 showing that *Cx. quinquefasciatus* differs from its sibling species *Cx. pipiens*.

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LACK OF GENETIC BOTTLENECKS DURING WEST NILE VIRUS INFECTION OF *CULEX PIPIENS QUINQUEFASCIATUS*

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The introduction and adaptation of arthropod-borne viruses (arboviruses) to new ecological niches pose an ongoing threat to human health. Understanding the evolution of arboviruses in the context of their transmission cycles and how this impacts viral emergence, adaptation and persistence is paramount to effectively minimizing public health risk. Introduced into North America in 1999, West Nile virus (WNV; *Flaviviridae*, *Flavivirus*) has adapted to local transmission cycles and is now considered endemic. Previous studies have shown that WNV exists within hosts as a genetically diverse group of competing viral variants, and that the complexity of the WNV quasispecies is greater in mosquitoes than birds. The mechanism(s) responsible for this observed difference and the impact of mosquito infections on viral evolution are unknown. Therefore, using WNV and *Culex pipiens quinquefasciatus* mosquitoes as a model system, we addressed the role mosquito infections have in shaping arbovirus populations. We offered *Cx. p. quinquefasciatus* mosquitoes an infectious bloodmeal containing a highly genetically diverse WNV population and quantified viral genetic diversity in three well-characterized stages (infection, dissemination, and transmission) from three mosquitoes at three time points (7, 14, and 21 days post infection). WNV RNA corresponding to the E-NS1 coding region (nt 1971-2928) was reverse transcribed, amplified with high fidelity polymerase and cloned. Subsequently, thirty clones from each sample were sequenced. Baseline levels of input viral genetic diversity were determined from whole-body mosquitoes recovered immediately post blood feeding. The genetic diversity of virus obtained from three freshly fed mosquitoes was exteremely high (0.170%). Further, on average, 14.7 haplotypes were identified with many of these haplotypes being shared among individuals. Examination of midguts from later timepoints revealed genetic restriction with a reduced number of haplotypes and an overall genetic diversity of 0.021%. Sampling of the legs (dissemination) and saliva (transmission) revealed that many of the input haplotypes that had been lost in the midgut were recovered, and viral genetic diversity was greater than in the midgut. Overall, these data suggest that the *Culex* mosquito midgut fails to impose a genetic bottleneck on WNV populations and that WNV genetic diversity accumulates during the course of an infection.

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INSIGHTS INTO ARBOVIRAL MUTANT SWARM DYNAMICS USING EXPERIMENTAL EVOLUTION OF FLAVIVIRUSES

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The rapid production of mutants created by the combination of high rates of replication and error prone nature of the RdRp of arboviruses results in a population of variants collectively called the mutant swarm. In order to fully understand how selection acts on these populations one must first fully describe the role of the mutant swarm both within and among hosts. Understanding the role of minority variants in viral fitness and plasticity is particularly important for arboviruses, which require replication in disparate hosts and tissues. In addition, an understanding how bottlenecks within and among hosts work to shape the arboviral mutant swarm is still lacking. Using *in vitro* and *in vivo*-passed strains of West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) we evaluated the importance of the mutant swarm in viral fitness, the distribution of variants in cell-adapted populations, the potential for strain complementation, and the size of the neutral bottlenecks within *Cx. pipiens* mosquitoes. Previous results have demonstrated that passed populations are highly adapted and, that the WNV mutant swarm is important in adaptive phenotypes. WNV viral diversity has been shown to accumulate at high levels with passage and adaptation to both mosquitoes and mosquito cells, while SLEV has not. Preliminary *in vitro* competition assays with WNV suggest that increases in strain co-infection in mosquito cells limit the relative fitness of the adapted strain, suggesting strain complementation in co-infected cells. In addition, preliminary results using equally fit WNV variants have allowed us to quantify the probability that minority variants will survive both dissemination throughout and transmission from *Cx. pipiens* mosquitoes given their starting concentrations in the population. Taken together, these results provide crucial insight into arboviral mutant swarm dynamics which have significant impact on the understanding of arbovirus adaptation, evolution, and epidemiology.

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PATTERN FORMATION IN THE DYNAMICS OF WEST NILE VIRUS AMPLIFICATION IN A TRANSMISSION HOT SPOT IN CHICAGO, U.S.A.

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Untangling the influence of host and vector diversity on West Nile virus (WNV) persistence and transmission is relatively complex given the large numbers of species and interactions. Although the complexity of these networks have been hypothesized to affect transmission, at times positively or negatively, the actual mechanisms that facilitate repeated outbreaks of WNV remain largely unknown. It has been suggested that "super spreaders," may be important in the WNV system, defined as a minority of individuals, or host species, that give rise to the majority of secondary infections. Here we examine this idea, using a series of computer simulations of pathogen transmission. Under most conditions, we find that cascades of WNV transmission are not solely driven by super spreaders, but rather by a critical mass of infected individuals. Although these data do not exclude the possibility that super spreaders are important, they suggest that making assumptions about their influence on pathogen dynamics requires careful testing.

1206

IL-10 AND PD-L1 IMPAIR THE T CELL RESPONSE IN THE CENTRAL NERVOUS SYSTEM DURING PERSISTENT WEST NILE VIRUS INFECTION

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West Nile virus (WNV) can lead to a persistent infection in humans and in several animal models. In mouse studies from our laboratory, WNV RNA persists in the central nervous system (CNS) for up to 6 months post-inoculation (p.i.), and infectious virus can be isolated from CNS tissues for up to 4 months p.i. We also showed that plasma cells in the CNS of WNV-inoculated mice secreted WNV-specific antibody for at least 16 weeks p.i. Additionally, we detected epitope-specific CD8+ T cells in the CNS for up to 16 weeks p.i., using a MHC class I dimer assay for a dominant WNV epitope (SSVWNATTA). We hypothesized that CD8+ T cells are

ineffective in clearing the virus from the CNS, resulting in viral persistence. We assessed the function of T cells using intracellular staining for IFN-γ and found that only 20% of the epitope-specific CD8+ T cells in the brain were able to secrete IFN-γ at 2 to16 weeks p.i. In contrast, approximately 95% of the epitope-specific CD8+ T cells in the spleen produced IFN-γ after peptide stimulation. Similar results with TNF-α production were obtained. We examined the possibility that the CD8+ T cells were inhibited by IL-10 and/or programmed death receptor ligand-1 (PD-L1). WNV-inoculated mice were treated with antibodies to IL-10 receptor (1L-10R) and/or PD-L1 every three days for five treatments starting at 2 weeks p.i. Three days after the last treatment, mice were sacrificed, and CD8+ T cell function and WNV persistence were compared to isotype antibody treated controls. The function of the epitope-specific CD8+ T cells was partially restored upon blockade of IL-10R and/or PD-L1. In addition, infectious WNV was found in two of nine mice treated with antibodies to both IL-10R and PD-L1 compared to eight of eight mice treated with isotype control antibodies (Fisher's exact test, *P*=0.002). In summary, these results suggest that CD8+ T cells are impaired in the CNS of mice due to IL-10 and PD-L1, and blockade of these molecules promotes viral clearance.

1207

KERATINOCYTES ARE CELL TARGETS OF WEST NILE VIRUS *IN VIVO*

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West Nile virus (WNV) is transmitted to humans through the bite of an infected mosquito at the skin. Langerhan cells, resident dendritic cells in the skin, are postulated to become infected with WNV and migrate to the draining lymph nodes. We hypothesized that WNV infects additional cell types in the skin because high levels of infectious virus are detected in both the skin and draining lymph node at 24 hour post-inoculation (hpi) and persist for at least seven days. The goal of this study was to identify the cell target(s) of WNV in the skin. We inoculated mice subcutaneously (SC) in the left rear footpad with WNV replicon particles containing a luciferase reporter and monitored luciferase activities in various tissues. Luciferase activity was detected in the skin at the inoculation site at 24 hpi and increased ~100-fold at 72 hpi. These results suggest that WNV infects non-migrating cells in the skin, which continually support virus replication. We inoculated mice SC in the footpads with WNV and performed immunohistochemical assays on the skin to determine the cell tropism of WNV. WNV-positive cells were found only in the epidermal layer of the skin of the WNV-inoculated mice at 96 hpi, and these cells were identified morphologically as keratinocytes. We inoculated mouse primary skin cells with WNV and analyzed the cells by flow cytometry for expression of WNV E antigen and cell surface markers - the pan-leukocyte marker CD45 and a keratinocyte marker K10. Approximately 4% of the skin cells expressed WNV antigen, and ~64% of these cells expressed K10, further confirming that mouse keratinocytes were susceptible to WNV infection. No WNV-positive cells expressed CD45, but they may not have been detected since there were low numbers of CD45-positive cells (~2.5%) in the skin cell population. In addition, primary human keratinocytes were permissive for WNV and supported virus production for at least six days. In summary, the skin is an initial replication site for WNV, and keratinocytes are cell targets *in vivo* in the skin.

1208

THE IMPACT OF A SENTINEL SITE MALARIA SURVEILLANCE PROGRAM ON IMPROVING CASE MANAGEMENT IN UGANDA

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Malaria surveillance is critical for monitoring trends in malaria morbidity and mortality, but can also be used as a tool for improving case management. The WHO now recommends ACTs for the treatment of uncomplicated *falciparum* malaria along with prompt laboratory confirmation in all patients suspected of malaria before treatment is started. From August 2006 - January 2007 we implemented a sentinel site malaria surveillance system at the outpatient departments of 5 government health centers in Uganda with a 6th site added in September 2008. This system was established by the Uganda Malaria Surveillance Project in collaboration with the Ministry of Health and Health Management Information System. Individual patient data is entered electronically onsite using a standardized case record form and sent monthly to a core facility using cellular technology. Through February 2010, a total of 397,407 patients were seen of which 54% (range 47-63% at the 6 sites) were suspected of having malaria. We compared data from the first two months of surveillance with data from the most recent two months (Jan-Feb 2010) to evaluate key indicators in malaria case management. The proportion of patients with suspected malaria for whom a laboratory test was done increased from 44% (range 32-64%) to 97% (range 93-99%). The proportion of patients with a laboratory test done who were appropriately prescribed an antimalarial drug (negative test not prescribed an antimalarial, positive test prescribed an antimalarial) increased from 64% (range 51-78%) to 94% (range 87-98%). The proportion of patients with a positive laboratory test who were prescribed an ACT increased from 50% (35-88%) to 74% (35-91%), although these results were highly variable across the sites. The implementation of sentinel site malaria surveillance system in the context of the existing government system was associated with dramatic improvements in utilization of laboratory services and rationale antimalarial treatment decision making. However, further improvement is needed in ACT prescribing practices.

1209

MATERNAL AND PLACENTAL MALARIA AND LOW BIRTH WEIGHT AFTER INTRODUCTION OF INTERMITTENT PREVENTIVE TREATMENT PROGRAM USING SULFADOXINE-PYRIMETHAMINE IN PREGNANT WOMEN IN BAMAKO, MALI, WEST AFRICA

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In 2006, the Malian government established a program for free insecticide-treated net (ITNs) and intermittent preventive treatment during pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) for pregnant women. In March to November of 2009, we conducted a cross-sectional study in periurban areas of Bamako, Mali to determine the malaria prevalence among pregnant women and their newborn children. We included 379 pregnant women aged 15 to 45 years. At delivery, malaria was diagnosed using peripheral thick smears in mothers and newborns, as well as

umbilical cord blood and placental blood. The prevalence of *P. falciparum* malaria was 2.4%, 1.6% and 0.5% respectively in mother, placenta and cord samples. Approximately 77% of our parturient were housewives. The illiteracy rate among this group was 72.3%. Of the 379 women, 72.8% had at least three prenatal visits, 82.8% had received at least one free ITNs, and 71.8% had received IPTp-SP during antenatal visit. Among them, 80.7% claimed to have complied with IPTp-SP. We observed a low birth weight rate of 12.1%. We did not find any congenital malaria. The prevalence of malaria in both mother and newborn has show a significant decrease in Bamako, compared with previous studies before the implementation of IPTp-SP policy in Mali. This study shows a high rate of coverage in use of IPTp-SP and ITNs correlated with low malaria prevalence in pregnant women.

1210

MALARIA RESURGENCE IN WESTERN KENYA IN THE ERA OF MALARIA INTERVENTIONS

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Despite significant increases in the supply of mosquito bednets since 2006, the link between interventions and their impact is not always clear in malaria endemic south Sahara Africa. To monitor the impacts of insecticide treated bed nets on malaria prevalence and mosquito density, we conducted monthly surveys in three sites in Western Kenya beginning in January 2007. Our results illustrate a resurgence of malaria in all three of our study sites (2 highland and one lowland) after an initial decline in 2006. Despite a high household bed net coverage rate of 50-80%, asymptomatic malaria prevalence and mosquito density have increased in all study sites since 2007, with an approximate 4-10 fold increase in *Anopheles* funestus and *An. gambiae* densities. Upon examination of households that used bednets, the majority of nets were in good condition, but only 60% were regularly used. Additionally, almost half of the bed nets were 4 or more years old, so their efficacy was waning. We also found a decrease in the prescription of ACT to treat malaria cases, even though ACT is the most effective drug at the moment. The increase in malaria prevalence and vector densities suggests that the current control methods are not sufficient and that future control effort should include a more intensive distribution of long-lasting insecticidal nets and the provisions for a sustainable supply of effective antimalarial drugs.

1211

SIGNIFICANT DECREASE IN ANEMIA IN AREAS OF VERY LOW MALARIA TRANSMISSION AFTER INTERRUPTION OF TRANSMISSION

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The potential effect of malaria control measures on prevalence of anemia in areas of low and unstable malaria transmission has not been well characterized. In the adjoining highland areas of Kapsisiywa and Kipsamoite, Kenya, areas of low and unstable malaria transmission, we assessed hemoglobin levels and frequency of anemia in a cohort of randomly selected asymptomatic individuals in May 2007 and July 2008 (Kapsisiywa, n=910, Kipsamoite, n=787). Widespread indoor residual spraying of insecticide led to a 12- month interruption of malaria transmission in both areas starting in April 2007, and none of the individuals in the cohort had clinical malaria between May 2007 and July 2008. In May 2007, anemia was common in both sites, with 57.5%, 21.7% and 22.7% prevalence in Kapsisiywa for individuals <5 years, 5-14 years and >15 years of age, respectively, and 47.2%, 19.3% and 17.5% prevalence in Kipsamoite for individuals <5 years, 5-14 years and >15 years of age, respectively. Fourteen months later, malaria interruption was associated with significant increases in hemoglobin levels and consequent decreases in the prevalence of anemia for individuals in Kapisisiywa <5 years of age (34.1% reduction of anemia, $P<0.0001$), 5-14 years of age (51.6%, $P<0.0001$) and >15 years of age (26.9%, $P=0.004$), and in Kipsamoite for individuals <5 years of age (33.7%, $P=0.001$). Insecticide treated net (ITN) use was infrequent in both sites (16.8% in Kapsisiywa, 11.3% in Kipsamoite), and was associated with an increase in hemoglobin level only in pregnant women. Even in areas of very low malaria transmission, anemia occurs frequently, particularly in children. Reduction or interruption of malaria transmission in these low transmission areas is associated with highly significant decreases in the prevalence of anemia.

1212

INTERMITTENT PREVENTIVE THERAPY IN THE POST-DISCHARGE MANAGEMENT OF SEVERE MALARIAL ANAEMIA IN PRE-SCHOOL CHILDREN; A MULTI-CENTRE RANDOMIZED PLACEBO CONTROLLED TRIAL IN SOUTHERN MALAWI

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Despite adequate in-hospital treatment with blood transfusions and antimalarials, young children admitted with severe malarial anaemia in malaria endemic regions in southern Malawi and western Kenya are at high risk of dying or being re-admitted in the first few months after discharge. They may represent a particularly vulnerable group due to a combination of parasite, host, environmental and socio-behavioural factors. In highly endemic areas, failure to clear the initial malaria infection and the acquisition of new infections in the first few months after discharge may negate the initial improvements in haemoglobin concentrations resulting from the blood transfusion. Interventions that result in radical cure of the initial infection and prevention of subsequent malaria episodes will provide a time-window in which the bone marrow can recover to restore haemoglobin levels. We have completed a randomized placebo controlled trial of the efficacy and safety of Intermittent Preventive Therapy post-discharge (IPTpd) in children aged 4-59 months admitted for severe malarial anaemia requiring blood transfusion in 4 hospitals in southern Malawi. Convalescent children who had successfully received a blood transfusion and quinine were randomized to receive either 3 treatment courses of artemether-lumefantrine given at discharge, and again at 1 and 2 months post-discharge, or a single treatment course of AL at discharge followed by placebo at 1 and 2 months. Children were followed for a total of 6 month to compare the rates of rebound severe malaria, severe anaemia, or death 1-3 months after discharge (the intervention period) and during the subsequent 3 months. Between June 2006 and Aug 2009, 1435 children aged 4-59 months were recruited from the Queens Elizabeth Central Hospital in Blantyre and 3 adjacent district hospitals within 1 hour drive from Blantyre; over 90% were followed-up successfully. Analysis is ongoing and study results will be presented.

1213

THE IMPACT OF INSECTICIDE-TREATED NETS ON ACQUIRED HUMORAL IMMUNITY TO *PLASMODIUM FALCIPARUM*

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Acquisition of immunity to malaria is attributed to frequent exposure to the parasite. Lack of exposure can result in loss of immunity, leaving individuals at higher risk for severe disease and death. Intensified malaria control interventions have played a major role in reducing malaria incidence and mortality. An important intervention has been the mass distribution of long-lasting, insecticide-treated bed nets (LLINs). Following implementation of these effective control programs, population immunity is expected to decrease. It is, therefore, important to monitor population immunity to measure the impact of control measures and anticipate future epidemics upon reintroduction of the parasite and vector. We investigated the relationship between the distribution of insecticide-treated bed nets and acquired humoral immunity to *Plasmodium falciparum* in a low malaria transmission area of Macha, Southern Province, Zambia one year after the widespread distribution of LLINs. IgG antibody levels were measured using an enzyme immunoassay against whole, asexual stage *P. falciparum* antigens derived from NF54 strain schizont cell lysate. Seropositivity was defined as an optical density value > 0.57 based on the mean plus three standard deviations from 10 individuals never exposed to malaria. Plasma was extracted from whole blood samples stored as dried blood spots. Samples were collected between March and November 2008 from 313 individuals residing in randomly-selected households in southern Zambia. 56.5% of the study participants were seropositive for antibodies to whole *P. falciparum* antigens. 31% of seropositive individuals reported sleeping under a bed net, compared with 39% of seronegative individuals. Within one year of introducing LLINs for malaria control in southern Zambia, we found no significant reduction in seropositivity to whole P. faliciparum antigens in those who reported sleeping under a bed net compared to those who did not. Further monitoring of changes in population immunity to malaria is warranted in regions implementing accelerated control efforts.

1214

A SURVEILLANCE SYSTEM TO MEASURE CHILDHOOD MORTALITY AND DRUG RELATED ADVERSE EVENTS IN THREE DISTRICTS IN SENEGAL

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A simplified Demographic Surveillance System (DSS) was established in three health districts in Senegal to measure childhood mortality and for monitoring the incidence of serious adverse events in an area where seasonal IPTc is being implemented. The DSS covers a population of 602,000 people living in rural and semi-urban communities served by 54 health posts in three districts, and includes the area of the long-standing Niakhar DSS. Births deaths and migrations, ITN use and hospitalizations are recorded in 6-monthly household rounds. Verbal autopsies are performed on all deaths in the population served by 12 of the health posts. Mothers of children under 10 years are issued with a DSS card bearing details of all the children in their care. This card is used to record any health interventions delivered at village level including malaria IPT, and to confirm child identity when the child visits a health facility. Incidence of malaria confirmed by rapid diagnostic test was recorded by passive case detection at health facilities. A cluster sample survey of the under-5 population was done at the end of the transmission season to estimate the prevalence of malaria parasitaemia and anaemia. In 2008 there was no evidence of the seasonal peak in deaths from September to November which was characteristic of the pattern of mortality in previous years. A dramatic reduction in under-5 mortality observed in the population maintained under long term surveillance in the Niakhar DSS is borne out in the much larger area covered by the new surveillance system. Incidence of malaria among children <5yrs was less than 1 per 1000. The prevalence of *P. falciparum* parasitaemia at the end of the 2008 transmission season was less than 4%. Very low incidence of malaria in 2008 was associated with substantial improvement in child survival and disappearance of the usual seasonal peak in under 5 deaths associated with malaria transmission, in a large rural population not previously kept under demographic surveillance.

1215

NOVEL MEMBRANE-ASSOCIATED PROTEIN KINASES IN TRYPANOSOMES

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Protein kinases modulate cellular responses to signals from the environment or from within the cell. Their ATP binding pocket renders them sensitive to small molecule inhibitors, making kinases attractive drug targets. *Trypanosoma brucei* possesses over 150 protein kinases; less than one-fourth of these have been studied in any detail. We have identified nine kinases that bear predicted transmembrane domains and hence could function to regulate processes spanning compartments within the parasite or between the parasite and its external environment. The presence of multiple transmembrane domains in the predicted structures of these kinases is unprecedented, indicating a novel means of signal transduction between the putative sensing and catalytic domains. Surprisingly, these kinases are localized to diverse structures within the parasite, indicating that they have varied cellular functions. One modulates the biogenesis of lipid bodies, organelles than function in intracellular lipid homeostasis. Two kinases localize to the secretory system, although these kinases do not resemble host cell ER kinases. Several of the kinases appear to be essential for bloodstream form viability, suggesting their potential use as drug targets.

1216

T CELL RESPONSES IN INDIVIDUALS WITH DISCORDANT *TRYPANOSOMA CRUZI* SEROLOGY

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a major cause of morbidity and mortality in Central and South America. Serological diagnosis of *T. cruzi* infection requires positive testing on two of three independent immunoassays. Geographic variations in the concordance of these diagnostic assays suggest that local parasite strain diversity may play a role in the strength of the anti-*T. cruzi* antibody response. Peru in particular has a high rate of discordant serology. To determine whether T cell responses could be used as a surrogate diagnosis in individuals with discordant serology, IFNγ production from peripheral blood mononuclear cells were stimulated with *T. cruzi* antigen was measured by ELISPOT assay. The studies were conducted in the

city of Arequipa and the town of LaJoya in the Peruvian Andes. Of the 103 subjects enrolled, 24 were seronegative, 53 were concordant by conventional serology ("seropositive"), and 26 were discordant by conventional serology ("discordant"). Peripheral blood mononuclear cells were stimulated with five different strains of *T. cruzi* - a laboratory strain (Brazil), two strains isolated from LaJoya, and two isolated from Bolivia - in order to determine if patients responded differently to parasites isolated from distinct geographic regions. Seronegative patients did not respond to the *T. cruzi* antigen. In the discordant and seropositive groups, the percent producing IFNγ in response to antigen stimulation was similar (approximately 67% in each group), and the frequency of IFNγ-producing cells was not statistically different in the discordant (mean = 39.7 spot-forming units/400,000 cells) and seropositive groups (mean = 42.7). Similar frequencies of IFNγ producing cells were detected regardless of the stimulating parasite strain, although responses were slightly lower in response to the Brazil strain. These data suggest that patients with discordant serology are likely infected with *T. cruzi* and that T cell responses may have utility as diagnostic tools in instances of inconclusive serology.

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ROLE OF HLA CLASS-II ALLELES (HLA DR AND DQ) IN PROTECTION/SUSCEPTIBILITY TO VISCERAL LEISHMANIASIS IN SUDAN

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The antigen-presenting molecules of the HLA class-II (DR, DQ and DP) have a possible role in the susceptibility to autoimmune and infectious diseases. In this study the possible role of these alleles in susceptibility/protection against visceral leishmaniasis (VL), a potentially fatal parasitic disease was investigated. Following informed consent, fifty four individuals with parasitologically confirmed history of VL together with forty individuals with no history of VL were enrolled. The volunteers were mostly from the Masalit (Nilo-Saharan; Zurga) and the Fellata (Bargo/Maba/Wadians) tribes of eastern Sudan. Genomic DNA was extracted from peripheral blood mononuclear cells and buccal wash deposit using the Phenol-Chloroform Isoamyl technique. The HLA-Class II alleles were identified using high resolution PCR-Sequencing Based Typing (PCR-SBT) technique. The DRB1*1101 allele was detected in 28.6% of patients with VL and in 17.7% of those in those with no history of VL (*p=0.5).* DRB1*1001-DQA1*0501-DQB1*0501/DRB1*1001-DQA1*0102- DQB1*0501 haplotypes were detected with highly significant frequencies in individuals who contracted VL compared to those who did not (*p=0.01-p=000012* respectively*).* On the otherhand DRB1*0804-DQA1*0101 haplotype was significantly more predominant in individuals who did not develop the disease compared to those who did (*p=000000)*In conclusion: the DRB1*0804-DQA1*0101 haplotype is a probable protection haplotype, while DRB1*1001-DQA1*0501-DQB1*0501/DRB1*1001-DQA1*0102-DQB1*0501 haplotypes are probable susceptibility haplotypes for visceral leishmaniasis in the some ethnic groups of Sudan. Study findings could be helpful in peptide vaccine development for VL.

1218

LEISHMANIA MEXICANA INFECTED *PHEBOTOMUS YUCATNICUS*: NO PRODUCTION BY LYMPHOCYTES-MACROPHAGES

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The Yucatan peninsula of Mexico is a well known endemic area of cutaneous leishmaniasis (CL) caused predominantly by *Leishmania (Leishmania) mexicana*. Studies of CL in the murine model (C57BL/6 infected with *L. major*) have documented the nitric oxide (NO) role in the elimination of the parasite through the classical macrophage activation by IFN-γ produced by CD4 Th1 lymphocytes. *Phebotomus yucatanicus* a primary reservoir of *L. mexicana* has been adapted to captivity and a colony for experimental studies has been developed. The main purpose was to compare NO production between cocultured macrophages and lymphocytes from *P. yucatanicus* infected with *L. mexicana* stimulated with or without SLA with and macrophages alone. Group A was inoculated with 1x102 promastigotes of *L. mexicana*; group B with 2.5x106 promastigotes; and group C inoculated with RPMI (n=14, per group). They were followed-up for 12 weeks to register clinical signs. At the end they were sacrificed to determine indirect nitrite oxide production employing Griess reaction. No one of the group A (subclinical) develops signs of CL, whereas in 13/14 (92.9%) of Group B show signs of CL. Nitrite oxide production was significative higher ($p<0.05$) in both Groups A and B (co-cultured lymphocytes and macrophages with or without SLA). Results support the employment of *P. yucatanicus* as a new experimental model to study CL.

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CHARACTERIZATION OF UNKNOWN PREDICTED GPI ANCHORED PROTEINS IN *TRYPANOSOMA BRUCEI BRUCEI*

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African trypanosomes of the *Trypanosoma brucei* complex undergo several differentiation steps during development in the tsetse fly, including differentiation to the procyclic form in the fly midgut, epimastigote forms in the proventriculus, and ultimately to mammalian infective metacyclic trypomastigotes in the salivary glands. Finally, metacyclics develop into the bloodstream form in the mammalian host. An in-silico screen of the *T. brucei* genome yielded 111 unknown proteins containing glycosylphosphatidylinisotol (GPI) anchor structures. GPI anchors typically bind proteins to cell membranes and as such, these hypothetical proteins may be expressed on the parasite cell surface. To determine if these unknown genes were preferentially expressed in particular developmental stages, expression analysis was performed on parasite infected salivary glands, proventriculus, and midguts, as well as bloodstream parasites. cDNAs were prepared, normalized, and expression levels were tested with gene-specific primers using a semi-quantitive assay. This revealed that the majority (66%) of the unknown genes were expressed in parasite stages infecting the proventriculus or salivary glands. Results were validated using quantitative PCR analysis. Twenty-one genes were determined to be specifically expressed in the salivary glands, and 15 of these were found to be expressed in the mammalian infective metacyclic form collected from tsetse saliva. A few of these genes were selected for further characterization at the RNA and protein level. Antibodies to the recombinant protein corresponding to one of these gene products have been used to demonstrate the expression profile of this protein in the both the immature (attached) salivary gland stages and free metacyclics. These

data can identify novel targets for blocking trypanosome transmission, and increase our broader understanding of this complex host-parasite interaction.

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INCREASED INTERFERON-Γ LEVELS OCCUR IN THE CEREBRAL SPINAL FLUID DURING THE CHRONIC PHASE OF *TRYPANOSOMA BRUCEI BRUCEI* INFECTION IN CATTLE

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Trypanosoma brucei brucei, one of the causative agents of nagana in animals, is a tissue invasive parasite. Monitoring of *T.b.brucei* infection in cattle is hampered by the lack of a clear parameter or marker that correlates with disease severity. In turn, this has made it difficult to measure benefits of disease intervention efforts such as immunization. *T.b.brucei* infection in cattle appears to mimic sleeping sickness in humans whereby it occurs in two phases, an acute or early phase where trypanosomes are readily seen in blood and a chronic or late phase where trypanosomes remain in tissues including the brain. In humans interferon gamma (IFN-γ) has been shown to be up-regulated in the cerebral spinal fluid (CSF) during the second stage of the disease and has been proposed as one of the markers of disease severity. However, the pathophysiological role of this cytokine in cattle has not yet been investigated. To substantiate this role, we carried out an experimental cattle infection under controlled indoor conditions. We analyzed the (IFN-γ) levels in the CSF during the acute phase and chronic phase and found that IFN-γ levels were elevated during the chronic phase of the disease. In addition to this, the levels of IFN-γ in the CSF were proportional to the dose used to initiate the infection. These data suggest that brain involvement plays a major part in the pathogenesis of *T.b.brucei* infection and IFN- γ is a marker for disease severity in cattle as has been proposed in humans

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DEVELOPMENT AND *IN VIVO* TESTING OF AN EFFECTIVE NOVEL DELIVERY VEHICLE FOR HETEROLOGOUS PROTEINS TO BOVINE ANIMALS

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We have developed a novel protozoan vehicle for the expression and delivery of heterologous proteins to bovines. Conditions for growth of the vehicle in a cell-free culture system have been developed and expression constructs engineered which permit the stable expression of a range of vaccine candidates or other proteins. These have been stably introduced and successfully expressed in the delivery vehicle, generating significant levels of the expressed protein. To evaluate the delivery-vehicle *in vivo*, the expression and immunogenicity of a known protective vaccine candidate against *Babesia divergens* has been evaluated. Inoculation in cattle resulted in the rapid establishment and stable maintenance of the vehicle over 9 weeks, without any adverse consequence for cattle welfare. Monitoring of the reactivity of the sero-response of the inoculated cattle by quantitative ELISA analysis demonstrated that specific antibodies to the delivered antigen were generated which progressively increased over time, achieving antibody titres known to be in the protective range. This novel delivery system offers potential as a flexible system, for example, for the generation of protective immune responses in cattle to a wide range of potential pathogens.

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COMPARATIVE GENOMIC HYBRIDISATION OF PHENOTYPICALLY DISTINCT *SCHISTOSOMA JAPONICUM* GEOGRAPHICAL ISOLATES

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Geographical isolates of Schistosoma japonicum from China and the Philippines exhibit a number of morphological and phenotypic differences, including pre-patent period, tegument topography, adult worm size, virulence and the subspecies of snail intermediate host infected. However, major genotypic differences have yet to be identified between the two strains. To address this deficit we have utilised microarray analysis to identify copy number variations within the genome of the two S. japonicum strains.

We have designed a Schistosoma japonicum custom microarray using EST data to examine 14,171 contigs. Since we intended to use the microarray to probe gDNA instead of mRNA, each probe was checked for relative specificity against the S. japonicum (Chinese) genomic supercontig assembly. We identified 8,310 microarray probes (of the total 14,171) with greater than 95% homology to only one region of the genome. This sub-group of probes present a high degree of specificity to the genome, since 60mer oligonucleotide probes with lower homology to the target sequence are known to result in significantly lower hybridisation efficiencies. The microarray platform was used to comparatively hybridise genomic DNA from Chinese mainland strain, and Philippine strain S. japonicum, in two colour experiments. Genomic DNAs from separate adult female or male parasites were used for comparison. We identified 306 contigs with differential hybridisation between the two S. japonicum isolates in both male and female parasites, which may represent a copy number difference. Validation and further characterisation of a subset of differentially hybridised genome regions is currently underway.It is hoped that these new data will provide insights into key aspects of the biology of this important human pathogen.

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GENETIC DIFFERENTIATION OF *SCHISTOSOMA MANSONI* LABORATORY POPULATIONS

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Changes in the composition of schistosome populations under differing environmental conditions, in differing hosts, and between developmental stages can be studied in parasite lab strains. A longitudinal study was performed with archived samples collected over 16 years from a population of *Schistosoma mansoni* maintained at CWRU and samples collected over 9 years from a related strain at the Biomedical Research Institute (BRI). Pooled samples of worms or cercariae were genotyped to obtain allele frequencies at 14 microsatellite loci and analyzed for the genetic differentiation measure *D*. Low levels of differentiation (first sample vs. other samples, $D<0.05$) were observed throughout the period examined for the BRI strain and for most of the CWRU samplings, with the exception of two time periods. The first was the result of an admixture event when the CWRU life cycle was supplemented with cercariae from an external source (first sample vs. pre-mixture, $D = 0.07$, vs. post-mixture, $D = 0.13$). The BRI strain was shown to be the external source (pre-mixture CWRU vs. BRI, $D = 0.35$; $D = 0.07$ post-mixture). The second event was a reduction in the number of mice used to maintain the CWRU life cycle, which increased genetic drift (prior to reduction mean *D* per generation = 0.002; 0.014 after reduction). To investigate how immunological background of the host influences differentiation, mice from 4 strains (C57, CF1, BALB/c, and BALB/c IFNg KO) which had been previously infected with *S. mansoni* were reinfected. Worms were

perfused, pooled by host strain, and genotyped. Negligible differentiation (mean $D < 0.01$) was seen between host strains. Very low differentiation was also observed between all life cycle stages in previously uninfected CF1 mice (for cercariae vs. worms, cercariae vs. eggs, and worms vs. eggs mean $D < 0.01$). Although the laboratory is a simpler and more controlled environment, these studies demonstrate the potential for monitoring the dynamics of field populations.

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PHYSICAL CONTACT MODULATES LARGE-SCALE GENE EXPRESSION IN *SCHISTOSOMA MANSONI* ADULT MALES AND FEMALES

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A continuous pairing with male is essential for *Schistosoma mansoni* female sexual maturation and to maintain reproductive activity and egg production. Our aim was to identify genes that are modulated by either physical contact or by the possible diffusion of proteins and/or hormones in the medium. Males and females from mixed infections were recovered by perfusion and triplicates of adult worms were maintained *in vitro* at 5 different culture conditions during 13 days (paired, separated males or females isolated from the opposite sex, females kept in the presence of males without physical contact, and couples remated for 7 days after separation for 6 days). We used oligonucleotide microarrays with ~44,000 probes. Statistical multiclass analysis of female data (paired, separated, females in the presence of male, and remated females) identified 1010 differentially expressed genes (FDR = 0.01%). This analysis revealed that remating restored gene expression profile of separate females to a similar profile as that of paired females. Furthermore, we observed that females in the presence of males, without physical contact showed a different gene expression from that of females in the absence of males. Statistical multiclass analysis of male data (paired, separated and remated males) identified 277 differentially expressed genes (FDR 1%). Males' gene expression was modulated by direct contact with females in paired couples, since paired male expression profile was significantly different from that of isolated males. Remating of separated males led to a gene expression profile similar to that of paired males. These results provide strong evidence for the influence of physical contact on large-scale gene expression of male and female adult worms. They also show that some genes, for which the change in expression does depend on the presence but not on direct contact between male and female, are possibly regulated by the diffusion of proteins and/or hormones released by the parasites in the medium.

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CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF *SCHISTOSOMA MANSONI* U6 PROMOTER

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The recent release of draft genome sequences of two of the major human schistosomes has underscored the pressing need to develop functional genomics approaches for these significant pathogens. The use of small interfering RNA (siRNA) molecules to achieve double stranded RNA-mediated interference (RNAi) has emerged as a powerful approach to sequence-specific gene knockdown. Vector based methods using siRNA expressing plasmid are used to induce RNAi in cells of eukaryotes and provide a long-term knockdown effect. We are developing vector based RNAi in *Schistosoma mansoni* using the U6 promoter to drive short hairpin RNA targeting reporter firefly luciferase. An upstream region of the U6 snRNA gene predicted to contain the U6 promoter was amplified from genomic DNA of *S. mansoni*, cloned and sequenced. A short hairpin RNA construct driven by U6 promoter targeting luciferase was engineered and cloned into pXL-Bac II (a *piggyBac* transposon donor construct). One day after mechanical transformation from cercariae, schistosomules of *S. mansoni* were exposed to the short hairpin construct by square wave electroporation. Short interfering RNAs (siRNA) specific for luciferase and a scrambled sequence short hairpin construct were used as positive and negative controls, respectively. 48h after exposure to short hairpin RNA constructs, schistosomules were electroporated with luciferase mRNA, harvested 3 hours later, and luciferase activity assayed. Short hairpin driven by *S. mansoni* U6 successfully knocked down luciferase activity by 20 to 50%. We are now transferring this technique to knock down endogenous genes in *S. mansoni* and to establish retrovirus and transposon based vector RNAi in transgenic schistosomes.

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TRANSCRIPTOMAL ANALYSES OF *SCHISTOSOMA MANSONI* PR1 TREATED WITH PRAZIQUANTEL

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Schistosoma mansoni is one of the most common etiological agents of human schistosomiasis and is estimated to infect more than 83 million people in 54 countries. Praziquantel (PZQ) is the least expensive, easiest to use and most readily available of all current anti-schistosomal drugs. One problem associated with PZQ treatment is that it does not kill schistosomes for a period of 2-4 weeks after they infect the human host. A second potential problem is the presence of drug resistance traits in natural populations of worms. As a result there is an urgent need to develop a new generation of anti-schistosomal drugs, a task that will be made easier by understanding the mechanism of action of PZQ. As yet, neither the molecule to which PZQ binds nor the means by which it kills mature schistosomes is known. The overarching aim of our study is to understand the molecular basis of PZQ sensitivity in *S. mansoni.* We hypothesize that PZQ sensitivity is a reflection of the differential expression of a gene that encodes either the PZQ binding partner or a downstream component of a biochemical pathway whose activity is influenced by PZQ binding to its partner molecule. Four and six week post infection (p.i.) *S. mansoni* PR1 have been treated *in vitro* with multiple sub-lethal as well as lethal doses of praziquantel. mRNA was extracted from replicate samples, cRNA prepared and labeled with Cy5 for transcriptomal analysis using a 44K *S. mansoni* microarray. All samples were compared against a common reference sample labeled with Cy3. Our initial analyses suggests that a number of genes associated with programmed cell death including cathepsins, Bax Interacting Factor 1 and death associated protein kinase (DAPK) are induced in 6 week (p.i.) praziquantel treated but not untreated schistosomes. DAPK has also been implicated in the phosphorylation of myosin light chains which has been reported to lead to vucuolation of cells and membrane blebbing - an often observed effect of PZQ on the mature but not juvenile worm tegument that may provide a more direct route to worm death.

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EXPRESSION LEVELS OF *SCHISTOSOMA MANSONI* MULTIDRUG RESISTANCE TRANSPORTERS INCREASE IN RESPONSE TO PRAZIQUANTEL AND ARE CORRELATED WITH REDUCED DRUG SUSCEPTIBILITY

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P-glycoprotein (Pgp) and the multidrug resistance-associated proteins (MRPs) are members of the ATP-binding cassette (ABC) superfamily of proteins involved in transport of toxins and xenobiotics from cells. These transporters likely play important physiological roles in the parasite's excretion of wastes and metabolites and provide attractive candidate targets for novel antischistosomal agents. Additionally, these

transporters are associated with development of drug resistance, and have been implicated in resistance to anthelmintics. We have previously shown that expression of *Schistosoma mansoni* Pgp (SMDR2) is altered in worms exposed to praziquantel (PZQ), the current drug of choice against schistosomiasis, and is expressed at higher levels in worms from isolates with reduced PZQ susceptibility. We have also shown that PZQ inhibits SMDR2, and also appears to be a substrate of SMDR2. Here, we examine the relationship between PZQ and SmMRP1, a *S. mansoni* homolog of mammalian MRP1, a transporter of anionic and GSH-detoxified compounds. SmMRP1 RNA is differentially expressed in adult males and females, which also show differences in the distribution of MRP1 immunoreactivity. Levels of SmMRP1 RNA increase transiently following exposure of adult worms to sub-lethal concentrations of PZQ. A corresponding, though delayed, increase in anti-MRP1 immunoreactive protein also occurs following exposure of worms to PZQ. PZQ-insensitive juvenile worms express higher levels of both SmMRP1 and SMDR2 RNA than mature adults, consistent with the hypothesis that increases in levels of schistosome multidrug transporters may be playing a role in development or maintenance of reduced susceptibility to PZQ. We are currently using molecular genetic and pharmacological approaches to define the physiological roles played by these transporters and to dissect the mechanisms by which they interact with PZQ and may modulate responsiveness to the drug.

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FORWARD GENETICS FOR *SCHISTOSOMA MANSONI*: QTL MAPPING OF OXAMNIQUINE RESISTANCE

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Schistosomes show heritable variation in biomedically important traits such as drug resistance, host specificity, and virulence. Our central aim is to develop forward genetic methods (i.e. linkage mapping) to identify parasite genes that underlie this phenotypic variation. This approach is well suited to *S. mansoni* as the lifecycle can be maintained in the laboratory and clonal propagation of parasites within snails generates large numbers of genetically identical parasites. To provide proof-of-principal that this approach is feasible and powerful, we have mapped a genome region that underlies resistance to oxamniquine (OXA). Resistance to OXA has arisen in nature, has a simple recessive basis and results in ~500-fold reduction in drug sensitivity. We crossed resistant and sensitive parasites (parents) and then crossed two F1 individuals to generate multiple F2 progeny, at each stage isolating individual parasite genotypes by infecting snails with single miracidia. We measured OXA-resistance by monitoring death of cultured worms following drug exposure and genotyped parents, F1 and F2 progeny using 64 microsatellite markers distributed at ~20cM intervals across the genome. As expected trait segregation in the cross was consistent with recessive inheritance as F1s were sensitive and ~25% of F2 progeny were resistant. We used the *S. mansoni* linkage map developed by our group to locate quantitative trait loci (QTL) underlying OXA resistance. We found a strong QTL (LOD = 5.43) on the p arm of chromosome 6 where microsatellite markers segregate closely with OXA resistance. The two parents of this cross have now been sequenced, simplifying fine mapping and identification of candidate genes. Successful identification of gene(s) that underlie OXA-resistance will provide insights into mode of drug action, allow development of modified compounds that kill resistant parasites, generate selectable markers for genetic manipulation, and set the stage for forward genetic analyses of a range of biomedically important traits including praziquantel resistance.

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BURDEN OF MALARIA IN HIV-POSITIVE PREGNANT WOMEN IN IBADAN, SOUTHWEST NIGERIA: AN ONGOING STUDY

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Pregnancy and HIV infection individually increase susceptibility to malaria. This gives the HIV-positive pregnant woman a double burden. Malaria transmission is intense and occurs all year round in southwest Nigeria. We evaluated the prevalence of malaria parasitemia by expert microscopy of Geimsa stained thick blood smear among HIV-positive (+ve) women at the PEPFAR Clinic and uninfected pregnant women at the antenatal clinic at booking both located at the University College Hospital Ibadan in southwest Nigeria. The prevalence of malaria parasitemia was 18.8% (24/128) among HIV +ve women and 5.9% (24/406) HIV -ve women (ρ = <0.0001, OR 3.673; 95% CI 2.004 -6.732). The geometric parasite density was higher among HIV +ve women but this was not significant. Mean hematocrit was significantly lower among HIV +ve women compared with their normal counter parts (30.37% ± 4.11 versus 34.5% ± 3.76; ρ <0.0001; *f-statistics* 107.129). The use of opportunistic infection chemoprophylaxis or antiretroviral therapy did not significantly influence the prevalence of malaria parasitemia. HIV -ve women booked significantly earlier than the HIV +ve women (21.26 ± 8.71 week versus 19.44 ± 7.64 weeks; ρ <0.029; *f-statistics 4.834)*. All socio-economic indicators (level of education of the women and their spouses, occupation of the women and their spouses, type of wall in accommodation, type of toilet facility, type of portable water and ownership of various gargets) were significantly (ρ <0.0001) lower among HIV +ve women. In conclusion, malaria exerts significant burden on HIV positive women in southwest Nigeria. These findings underscore the need for an adequate regimen of IPTp as well as other malaria control efforts (ITN, IRS and prompt case management) specifically targeted at HIV +ve women.

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HUMAN IMMUNODEFICIENCY VIRUS (HIV) TYPES WESTERN BLOT PROFILES AS SURROGATE MARKERS OF HIV DISEASE PROGRESSION

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A nested case-control study within a PMTCT cohort of antiretroviral therapy naive pregnant women. HIV-1/-2 Western blot test and surrogate markers of HIV disease progression were determined at 36 gestational weeks. Among the 64 pregnant women with HIV infection 98.4 % had pure HIV-1 infections and one woman (1.6%) had dual HIV-1/HIV-2 infections, with 100% band reactivity to both the envelope and polymerase proteins. However reactions to the *gag* core protein genes varied, being 100%, 90%, 70% and 63% for the p24, p17, p39 and p55 respectively. Lack of antibody reaction to *gag* p39 protein was significantly associated with disease progression among women with chronic HIV-1 infection as demonstrated by the presence of lymphadenopathy, anemia and higher viral load, p=0.010, 0.025 and 0.016 respectively. Although not statistically significant, women who had *gag* protein p39 missing were 1.4 times more likely to transmit the virus to their infants. In conclusion, absence of *gag* p39 and *pol* gene bands was significantly associated with disease progression and sero-conversion respectively. This data emphasises the importance of considering both the *env* and *pol* genes

proteins in the intepretation of positive Western blot HIV test results. Band profiles and simple laboratory tests like differential counts together with clinical symptoms could be useful in establishing and evaluating disease progression prior to antiretroviral therapy.

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MARKED REDUCTION IN THE PREVALENCE OF MALARIA AND ANEMIA IN HIV-INFECTED PREGNANT WOMEN TAKING COTRIMOXAZOLE WITH OR WITHOUT SP-IPT IN MALAWI

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HIV-infected women are at risk for both malaria and other opportunistic infection. Current WHO guidelines recommend cotrimoxazole for all HIV-infected pregnant women. However, it is not known whether this regimen is as effective as sulfadoxine-pyrimethamine (SP) intermittent preventive therapy (IPTp) in preventing malaria. Here, we compare the effectiveness of cotrimoxazole and SP-IPTp in protecting against malaria in a cohort of HIV-infected pregnant women in Malawi. From 2005 to 2009, we conducted a cross-sectional study of HIV-infected pregnant women attending routine antenatal services at Thyolo district hospital, Malawi. Peripheral blood was tested for anemia (hemoglobin<11g/dl) and malaria by both microscopy and PCR. We also collected data on use of anti-malaria interventions and other potential risk factors. CTX prophylaxis for HIV-infected pregnant women was introduced as policy in 2007, but implementation problems resulted in some women receiving CTX alone, SP-IPTp or both. We enrolled 1,142 women of whom 1,121 had data on CTX and/or SP-IPTp intake. Of these, 49.7%, 29.8%, and 15.4% reported taking SP-IPTp only, CTX only and SP-IPTp plus CTX respectively. Compared with women taking SP-IPTp only, those taking CTX (with or without SP-IPTp) were less likely to have microscopic malaria (OR, [95%CI]: 0.09, [0.01-0.66] and 0.43, [0.19-0.97] respectively) or PCR-detected malaria infections (OR 0.24; 95%CI 0.10-0.62 and 0.44; 95%CI 0.25-0.78 respectively) or anemia (PR, [95% CI]: 0.67, [0.54-0.83] and 0.72, [0.61-0.83] respectively). In conclusion, in HIV-infected pregnant women, CTX with or without SP-IPTp reduces the risk of malaria and anemia compared to SP-IPTp only. Longitudinal studies need to assess the effects of these drugs on birth outcomes and toxicity.

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INCIDENCE OF MALARIA EPISODES IN WEST AFRICAN ADULTS HIV-1 INFECTED PATIENTS EXPOSED OR NOT TO COTRIMOXAZOLE: MALHIV COHORT STUDY

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A key issue of the interrelation between HIV/AIDS and malaria is the impact of cotrimoxazole chemoprophylaxis on the natural history of malaria among HIV-infected patients. The objective of this study was to determine the incidence of malaria among HIV-1 infected patients, receiving daily cotrimoxazole or not. West African adults HIV-1 infected patients, receiving or not cotrimoxazole were enrolled in the 30 months MALHIV cohort study. Patients were divided into two groups: Group A CD4 < 350/mm^3 exposed to cotrimoxazole and group B CD4 ≥ 350/ mm^3 non exposed to cotrimoxazole. The cumulative incidence of clinical and parasitological malaria episodes was evaluated at baseline, every six months and in case of fever. Comparison of incidence in the two groups was used to assess the influence of cotrimoxazole primary chemoprophylaxis. At inclusion, the 548 enrolled patients [mean age=35.1 year, sex ratio: 0.29] had a CD4 mean of 198/µl and a viral load mean of 5, 2 $\log_{10}$ 368 patients (68%) were included in group A and 168 (32%) in group B, of whom 23 (4%) were lost to follow-up and 11 (2%) died. 325 patients received antiretroviral therapy. Asymptomatic parasitemia was diagnosed in 13 patients (2, 5%). Overall 35 malaria episodes (6.4%) occurred in 30 patients (5 in group A, 30 in group B). One case of severe malaria was noted. Malaria episodes were less frequent in group A. (1.3% versus 17% [RR= 0.03, IC=0.07-0.19, P< 0.05]). Fever episodes were less frequent in group A. (18, 9 %versus 63% [RR= 0,45; IC=036-0, 55 and P<0.05]. In conclusion, there is a trend of protection against malaria and fever episodes in HIV infected patients receiving cotrimoxazole in stable malaria transmission areas, even at lower CD4 cell counts.

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UTILITY OF PARACHECK-*PF*™ MALARIA RAPID DIAGNOSTIC TEST FOR THE DIAGNOSIS OF MALARIA AT POINT OF CARE AMONG ADULT HIV-POSITIVE PATIENTS IN IBADAN SOUTH WESTERN NIGERIA: AN ONGOING STUDY

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Malaria and HIV are major public health problems in sub-Saharan Africa. Febrile illnesses occur frequently among HI+ve patients and these febrile episodes are often first treated presumptively as malaria in endemic areas. In other to eliminate unnecessary treatments; reduce drug-drug interactions and the chances for emergence of drug resistant *Plasmodium,* the World Health Organization recommends a parasite-based diagnosis of malaria as much as possible. We evaluated 301 HIV+ve patients with febrile episode suspected of being malaria by expert microscopy and Paracheck *pf*™, (a histidine-rich protein-2 based malaria rapid diagnostic test, Orchid Biomedical Systems, Goa India) at the PEPFAR Clinic of the University College Hospital Ibadan, Nigeria. The mean age was 36.7 years (±9.2). About 80% (240/301) enrollees were female and 26.9% (81) had received various antimalarial drugs in the preceding two weeks. The prevalence of malaria parasitemia was 18.9% (357/301) by microscopy of thick blood smear with a geometric mean parasite density of 470/µL (range 39 - 204,000/µL). The prevalence of parasitemia by Paracheck *pf*™ was 18.5% (55/297) and four samples which were all blood smear negative gave indeterminate result for RDT testing. Sensitivity and specificity of Paracheck *pf*™ when compared with microscopy at all parasite densities were 54.4% and 90% while corresponding figures at parasite densities ≥ 200/µL were 93.9% and 91.7% respectively. Negative and positive predictive values at all parasite densities were 90% and 54.4%. Corresponding negative and positive predictive values for parasite density ≥ 200/µL were 99.2% and 58.5% respectively. The overall test accuracy was 83.2% while test accuracy at parasite density ≥ 200/µL was 91.9%. In conclusion, Paracheck *pf*™ malaria rapid diagnostic test accurately ruled malaria "in or out" at the point-of-care, facilitating appropriate clinical management and averting unnecessary antimalarial therapy.

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DIAGNOSIS OF IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME IN SCHISTOSOMIASIS PATIENTS UNDERGOING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY IN WESTERN KENYA

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The immune reconstitution inflammatory syndrome (IRIS) is a frequent complication seen in HIV-TB co-infected patients during successful highly active antiretroviral therapy (HAART). IRIS is common in resource-limited countries where the underlying prevalence of opportunistic infections is high, and where patients initiating HAART are more likely to have advanced immunodeficiency. We conducted a clinical study to investigate the immunopathogenesis, clinical aspects and manifestation of IRIS in HIV-schistosome co-infected patients undergoing HAART along the shores of Lake Victoria in western Kenya. Adults who were exposed to snail-infested lake water and are dually infected with HIV and *Schistosoma mansoni* were enrolled into the study. Baseline and follow-up indicators included CD4 counts measured by four-color flow cytometry, viral load measured by PCR, *S. mansoni* egg counts by the Kato-Katz method and evaluation of liver pathology by ultrasound. The prevalence of *S. mansoni* infections in this population was over 90% while that of HIV infection was 16%, and 67.9 % of those that were HIV positive had dual infections. Only 4% had detectable liver pathology at baseline. The intensity of schistosome infection had no influence on either the percentage of T helper cells (CD4+) ($p = 0.3640$) or the frequency of viral load copies ($p = 0.8470$). However, individuals with *S. mansoni* infection only had significantly higher mean egg production compared to those with dual infections. Clinical monitoring of dually infected patients getting started on HAART is ongoing to determine the case definition and the prevalence of IRIS.

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ALANYL-GLUTAMINE SUPPLEMENTATION PROTECTIVE EFFECTS ON NELFINAVIR-INDUCED INTESTINAL EPITHELIAL INJURY

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HIV protease inhibitors (PI) remain a crucial component of highly active therapy (HAART) contributing to reduce mortality and morbidity related to Human Immunodeficiency Virus (HIV) infection. However, discontinuation of therapy remains an important issue, which may be related to various side-effects, especially diarrhea. The aim of this study was to evaluate the effects of nelfinavir, an HIV PI, and of alanyl-glutamine supplementation, on intestinal cell migration, proliferation, apoptosis, necrosis, using a rat intestinal cell line, IEC-6, and on crypt depth, villi length, mitotic index and apoptosis using swiss mice. Migration was evaluated at 12 and 24h after injury, using a wounding assay. Proliferation was measured indirectly at 24 and 48h, using tetrazolium salt WST-1. Apoptosis and necrosis were measured by flow cytometry, using the Annexin V assay. We measured intestinal morphometry, mitotic index and apoptosis in mice following a seven day treatment with 100mg/kg of nelfinavir, given orally. Nelfinavir decreased IEC-6 cell proliferation and migration and increased apoptosis and necrosis. Alanyl-glutamine supplementation enhanced intestinal cell migration both at 12 and 24h and proliferation at 24 and 48h, but did not decrease apoptosis and necrosis after nelfinavir treatment. Nelfinavir decreased duodenal villus height and villus surface area, which was reversed with the addition of alanyl-glutamine and increased apoptosis. Alanyl-glutamine also increased crypt depth in the duodenum, jejunum, and ileum and duodenal mitotic index in nelfinavir-treated mice. In conclusion, alanyl-glutamine reversed nelfinavir-induced intestinal epithelial damage *in vitro* and *in vivo*. Alanyl-glutamine supplementation may be beneficial in the prevention or treatment of diarrhea induced by PIs during HAART.

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A NATIONAL MALARIA TRAINING MODEL FOR ACHIEVING RATIONAL USE OF ANTIMALARIAL MEDICINES IN SUB-SAHARAN AFRICA

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Presumptive treatment of malaria is a common practice in Africa resulting in widespread overtreatment, misuse of costly artemisinin-combination therapy (ACT), and inappropriate treatment of non-malarial febrile illnesses. Accordia Global Health Foundation's Integrated Management of Malaria (IMM) course and an on-site training program using Rapid Diagnostic Tests (RDT), developed in partnership with the Infectious Diseases Institute (IDI) at Makerere University and others, target multidisciplinary clinical teams and achieve substantial declines in the unnecessary use of antimalarials. Research conducted in partnership with the Uganda Malaria Surveillance Project (UMSP) demonstrated that the improvement was achieved at no detriment to patient health outcomes. Recently-issued WHO guidelines for malaria treatment will substantially increase demand for training in correct laboratory and RDT diagnosis of malaria, for the many African countries who will strive to achieve them. To enable high quality training delivered cost-effectively to a national scale, Accordia complemented its IMM and RDT training programs with field-based and peer-facilitated versions of those courses, led by graduates of the IDI-based courses at a fraction of the cost of centralized training. Following completion of additional coursework at IDI, IMM graduates conducted "Cascade Training" and achieved reductions in antimalarial use similar to those shown in IDI-based training: in three sites that received Cascade Training, the probability of an antimalarial drug being prescribed to someone who had a negative blood smear decreased by 28%; there was a 25% decline at eight sites that received the IDI-based training. The resulting National Malaria Training Model, reinforced by periodic site visits by Mobile Teams and a toll-free call center for clinical consultation, presents a cost-effective approach to build national capacity in the appropriate diagnosis of malaria and rational use of antimalarial medicines in sub-Saharan African nations.

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LARGE-SCALE IMPLEMENTATION OF INTERMITTENT PREVENTIVE TREATMENT IN INFANT WITH SULFADOXINE PYRIMETHAMINE FOR MALARIA IN RURAL AREAS OF HIGH TRANSMISSION IN SENEGAL

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Intermittent Preventive Treatment in infants (IPTi) is a new promising intervention for malaria control in Sub Saharan Africa which can be delivered through the existing routine Expanded Programme on Immunization (EPI). Although the efficacy of IPTi is proven, there is

limited economic evaluation data on scaling up IPTi using sulfadoxine pyrimethamine (SP) as a strategy for malaria control in Senegal. Implementation scale-up cost was calculated by estimating IPTi incremental costs in the start-up year and in subsequent years. Costs included were the financial costs of delivering IPTi to infants (drugs and equipment) and of programme activities. In recurrent years, because a survey in IPTi areas showed high acceptability and health care worker knowledge, programme costs included only IPTi administration and safety surveillance. To estimate IPTi cost-effectiveness (IPTi net cost per case of malaria averted, per death averted, per year of life saved, and per disability adjusted life years) we calculated the intervention's economic costs in recurrent years using a pooled efficacy analysis. A time and motion study was also conducted to assess staff time on IPTi delivery and the associated costs. In order to delivery IPTi-SP to 17,500 infants the total amount of the financial expenditure of programme implementation was $40,753.

IPTi incremental financial costs on start up year (3.31 $US/infant) were substantially higher than in recurrent years (1.043 $US/infant). In startup years communication activities mobilized the most important expenditure (30%) meanwhile, half of the total programme resources is allocated to capacity building development of professional staff. In routine, the part of programme costs was US$ 0.81 per infant, and patient costs (drug and utensils) only 23.7 US cent/infant. The time needed to administer IPTi was an average of 2.19 min/child, representing 7% of the time spends by health workers in immunization clinics. The net cost per averted case of malaria was $22.11.The cost per death averted was $ 447, per year of life saved $ 23.84 and per DALYs $ 25.39. In conclusion, IPTi with SP through EPI can be scaled up successfully at a low cost. Using pooled efficacy results from previous IPTi trails we can see that it is a highly cost effective intervention in the study sites selected. But, the IPTi cost-effectiveness in the other areas in Senegal where malaria transmission is not high, worth assessing.

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ARTEMETHER-LUMEFANTRINE VERSUS DIHYDROARTEMISININ-PIPERAQUINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA: LONGITUDINAL OUTCOMES IN A COHORT OF YOUNG UGANDAN CHILDREN

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There are limited comparative data on the long term effects of artemisinin-combination therapies (ACTs) for the treatment of malaria. In a cohort of young Ugandan children living in a highly endemic area, we previously reported that artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) were both highly efficacious, but DP was associated with a longer post-treatment prophylactic effect. Here we aimed to compare longitudinal outcomes in this cohort. Children were given a long-lasting insecticide treated bednet (LLITN) at enrollment and followed for all their healthcare needs. 305 children with a median age of 10 months (range 4-40) were randomized to AL (n=155) or DP (n=150) at the time of their first episode of uncomplicated malaria. The same treatment was given for all subsequent episodes of uncomplicated malaria and episodes of complicated malaria were treated with quinine. After randomization, children were followed a median of 22 months and a total of 2,592 treatments for malaria were given. The incidence of malaria following randomization was higher in the AL arm (5.44 episodes PPY) compared to the DP arm (4.74 episodes PPY), although this difference did not reach statistical significance (IRR=1.13, p=0.06). After randomization, only 27 treatments with quinine (1%) were given for complicated malaria (17 with convulsions, 10 with severe anemia). The incidence of complicated malaria was significantly higher in those randomized to AL compared to DP (IRR=4.84, p=0.006). All 6 early treatment failures were due to the development of complicated malaria within 2 days of initiating treatment with AL. There was one death due to malaria in a child randomized to AL. In this cohort of young children living in a highly endemic area the incidence of malaria was very high despite the use of LLITNs. However, treatment with AL or DP was highly efficacious and the incidence of complicated malaria was relatively low. DP was associated with a trend towards a modest decrease in the incidence of malaria and may lower the risk of complicated malaria compared to AL.

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COMMUNITY BASED MALARIA CONTROL IN SARAYA, SOUTHEASTERN SENEGAL

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In 2009, Senegal's National Malaria Control Program (NMCP) initiated a new Home-based Malaria Management (HMM) program, in which community health workers (CHWs) are trained to diagnose *Plasmodium falciparum* with rapid diagnostic tests and to treat appropriate patients with artemisinin-based combination therapy (artesunate + amodiaquine). The intention is to provide care to 30 villages of 200 to 500 inhabitants, which are all located 5 to 90 km from the nearest government health facility in the Saraya district of southeastern Senegal. The NMCP designed and implemented the 3-day training, which covered: modes of transmission and strategies for prevention; the clinical presentation of malarial infection; and the protocol to diagnose and treat uncomplicated malaria. The training was conducted in French and translated into Malinké. The training materials included a PowerPoint presentation and an instructional illustrated packet. This study evaluated the effectiveness of the training of CHWs. The same self-administered printed multiple-choice questionnaire was administered before and after the training. The questionnaire included questions to assess modes of transmission and strategies for prevention, the clinical presentation of malarial infection, and the protocol to diagnose and treat uncomplicated malaria. Scores in these three areas were determined. Before and after scores were evaluated using a two-tailed t-test. A P-value ≤ 0.05 was considered significant. Twenty-six CHWs participated in the training. Six CHWs were unable to read the training materials or questionnaires. The CHWs who could read and write assisted the others. The mean before and after scores were 52% vs. 73% (p=0.02) for CHWs understanding of transmission and prevention; 37% vs. 47% (p=0.21) for recognition of the clinical presentation of malaria; and 52% vs. 75% (p<0.01) for the ability to understand and carry out the HMM protocol. This study suggests that the training was successful in improving CHWs knowledge of transmission and prevention and ability to follow HMM protocol. Improvements should be made in the training the CHWs in disease recognition and future training should take the literacy level of CHWs into account. Further studies should be done to see how this knowledge translates into field practice and the need for training to reinforce these skills.

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PHARMACOVIGILANCE AND ANTIMALARIAL TREATMENT IN UGANDA: RESULTS AND EXPERIENCE FROM A PILOT ACTIVE SURVEILLANCE SYSTEM

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The safety of artemisinin combination therapies (ACTs) under large-scale operational use is not fully known. Continued careful monitoring after registration and release onto the market is therefore essential to establish ACT safety under routine conditions. An effective reporting system is a key requirement. Adverse event (AE) reports can be collected though passive spontaneous reports by users and prescribers and through active surveillance of patients receiving treatment. Active follow-up of patients to prospectively assess for AEs after treatment is ideal for detecting safety signals much more quickly and for estimating the incidence of AEs. Between November 2008 and October 2009 we actively followed up 842 patients treated with an antimalarial at public health facilities to assess the incidence of AEs at intervals until 28 days after treatment. Although artemether-lumefantrine (AL) is the recommended first-line treatment of uncomplicated malaria in Uganda, frequent stock-outs of AL resulted in use of many alternative antimalarials during the study period, including chloroquine, amodiaquine, sulfadoxine-pyrimethamine, artesunate monotherapy and quinine. Of 443 AEs reported, 271 (61%) were reported in association with ACT use. Cough, loss of appetite, flu-like symptoms, abdominal pain and weakness/fatigue were commonly reported but no serious AEs were reported. Drug stock-outs at the public health facilities forces patients to fill prescriptions outside formal systems, and affected the quality of collected information. Frequent changes of participants' addresses as well the high resource intensity of the system were major challenges. Effective active adverse event surveillance would require strengthening existing health infrastructure including health worker skills, diagnostic capacity for investigating and managing suspected adverse drug reactions and a good patient referral system.

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PHARMACOVIGILANCE OF ARTEMETHER-LUMEFANTRINE IN PREGNANT WOMEN FOLLOWED UP UNTIL DELIVERY IN RWANDA

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Antimalarial drugs that are considered safe during pregnancy become increasingly ineffective. Many countries shifted their treatment guidelines to artemisinin combination treatment (ACT). WHO recommends ACT for uncomplicated *falciparum* malaria in the second and third trimester of pregnancy. In Rwanda, the current policy recommends treatment with artemether-lumefantrine (AL) for malaria during the second and third trimester of pregnancy. However, safety data on ACTs in pregnancy are still limited and more data are warranted.In this proactive pharmacovigilance study we followed pregnant women treated with AL and a matching control group (CG) of healthy pregnant women not exposed to AL in the current pregnancy. Routine antenatal and peripartum data, pregnancy outcomes, congenital malformation and adverse events (AEs) were captured during acute episodes of malaria, routine ante-natal visits, at delivery in the health center or within 48hrs after a home delivery, by history taking and physical examination of the mother and the newborns at delivery. The data for 1783 women (AL n=881; CG n=902) showed: abortions: AL 8 (0.9%), CG 5 (0.6%); perinatal mortality:AL 28 (3.2%), CG 20 (2.2%); comprised stillbirth :AL 25 (2.8%), CG 16 (1.8%); neonatal death ≤7 days after birth :AL 3 (0.3%), CG 4 (0.4%), premature delivery AL :6 (0.7%), CG 3 (0.3%) and congenital malformations: AL 1 (0.1%), CG: 2 (0.2%). A total of 73 AEs were reported (AL 43 (4.9%), CG 30 (3.3%). The most common AEs were still birth. These data showed that the two arms were comparable in terms of pregnancy outcomes and AEs and did not show any specific safety concerns with AL treatment in pregnancy. The slight difference in AEs and pregnancy outcomes between the groups may be due to malaria itself but needs to be assessed further.

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EFFECTS OF STEADY-STATE LOPINAVIR/RITONAVIR ON THE PHARMACOKINETICS OF QUININE IN HEALTHY VOLUNTEERS

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Many antiretroviral and antimalarial drugs share overlapping cytochrome P450 (CYP) -mediated metabolic pathways, indicating potential for drug-drug interactions and clinically important changes in the efficacy or toxicity of these drugs. We conducted a phase I, multiple dose, sequential design pharmacokinetic study to assess the impact of chronic administration of ritonavir-boosted lopinavir (LPV/r), a fixed-dose combination of two HIV protease inhibitors increasingly used in developing countries, on the safety and pharmacokinetics of quinine sulfate in healthy volunteers. Thirteen adult volunteers received a single oral dose of quinine sulfate on Day 1 and Day 15, and LPV/r on Days 4-17. Blood samples were collected to measure plasma concentrations of total and free quinine and 3-hydroxy-quinine metabolite using HPLC-florescence detection. Adverse effects were common, mild-moderate in severity, and self-limited. EKG changes were seen in 7 of 13 participants. Day 15 to Day 1 geometric mean ratios (90% confidence interval) showed: free quinine area under the curve (AUC) 0.40 (0.74-0.22), maximal concentration (Cmax) 0.40 (0.58-0.27), terminal half-life (t1/2) 0.67 (1.0-0.45), apparent volume of distribution (V/F) 1.66 (1.15-2.40), apparent total clearance (Cl/F) 2.5 (1.34-4.65); total quinine AUC 0.49 (0.47-0.51), Cmax 0.51 (0.50-0.52), t1/2 0.79 (0.74-0.85), V/F 1.61 (1.59-1.63), Cl/F 2.0 (1.96-2.13). The addition of LPV/r caused a significant decrease in exposure of both total and free quinine and 3-hydroxyquinine, a decrease in protein binding and metabolite/parent ratio, and an increase in the apparent volume of distribution and total clearance. The study showed significant modification in quinine pharmacokinetics in the presence of LPV/r through a complex interplay of effects on CYP3A4, p-glycoprotein, and protein binding. Further studies are needed to assess changes in quinine pharmacokinetics and pharmacodynamics in individuals with malaria-HIV co-infection who are taking LPV/r-containing antiretroviral therapy.

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SUSTAINABILITY OF INTERVENTION FOR HOME MANAGEMENT OF MALARIA: THE NIGERIAN EXPERIENCE

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An intervention was carried out between2005-2007 to improve home management of malaria using artemeter lumefantrine [Coartem®] in Ona-Ara Local Government Area (LGA) of Oyo State, Nigeria. At the expiration of the project, the community was implored to sustain the program and provide support to the trained community based medicine distributors (CMDs). This study evaluated the sustainability of the HMM project two years after its expiration. A community-based study was

conducted among CMDs and stakeholders in Ona-Ara LGA. A total of 12 FGDs was conducted among CMDs, mothers of children aged 0-5 years and community members. Ten Key Informant Interviews were conducted with community leaders, Primary Health Care Coordinator, Rollback Malaria Manager, and PHC unit staff. After transcribing and word processing the data, Word Atlas Ti software was used to analyse data according to themes. Most of the FGD participants and Key Informants indicated that they were aware of the home management of malaria project in the area. In fact, there was consensus that communities selected their CMDs who distributed Coartem®. Data revealed that the treatment guideline poster given to households was of good assistance for treating children with malaria and some participants could still recapitulate the required dosages for treating malaria among children of different ages. The participants were of the opinion that the occurrence and severity of malaria has reduced in the area. While some CMDs have abandoned the assignment, few continue to provide care to febrile children and distribute Coartem® when available as their own contribution to the good of the community. Source of Coartem® was still the nearest government health facilities but supply was irregular and hindered by incessant transfer of health workers who were acquaintances on the project. All the CMDs mentioned they did not receive any support from the community. Most of the participants wanted the project to continue to support treatment of malaria in the community. In conclusion, while the project has proved to be life transforming, community support for CMDs is a serious challenge to sustainable HMM.

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NOVEL SYNTHETIC OZONIDE OZ439: TOLERABILITY AND PHARMACOKINETICS IN HEALTHY VOLUNTEERS

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OZ439 is a synthetic ozonide (1,2,4-trioxolane) that has potential value as a peroxide antimalarial agent. The clinical protocol design of a randomised, double-blind, placebo controlled study combined the multi-objectives of sequential single (SRD) and multiple dose rising (MRD) as well as a food-effect sub-study. The study was conducted under US-IND. Dose levels of OZ439 investigated in the SRD ranged from 50mg to 1600mg and in the MRD ranged 200mg to 800mg. The food effect (FE) component was studied with 800mg OZ439 in a placebo cross-over design. Healthy male and female subjects aged 18-55 years were recruited for the study. A total of 63 subjects (26 in SRD, 13 in FE, and 24 in MRD) were enrolled. Dose escalation was subject to approval from a Safety Review Board. Safety assessments consisted of vital signs, 12-lead and Holter ECG, laboratory tests, adverse events (AE), audiometry/BAEP parameters. Samples were taken for assay of OZ439 and its metabolites from blood drawn up to 96 hours post drug administration. OZ439 was shown to be safe and well tolerated at the doses studied in the SRD, MRD and fed/fasted state. The most common AEs for OZ439 (2 or more subjects) were headache, nausea, diarrhea, constipation, blood CPK increase, flushing, throat irritation and gastrointestinal hypermotility. One subject dosed with OZ439 (800mg) was prematurely discontinued due to syncope vasovagal (with ECG changes). There were no significant changes in laboratory values or mean changes in vital signs, audiometry/BAEP and ECG/QTc. On SRD and MRD, exposure to OZ439 and its metabolites (based on Cmax and AUC) increased with increasing dose, this being dose-proportional. Following MRD, mean plasma concentration-time profile of OZ439 was characterised by a median tmax of 3.00 hours followed by a multi-phase elimination. Mean t1/2 of OZ439 ranged from 37.8 to 41.7 hours. The plasma concentration-time profiles of the metabolites resembled that of parent compound. Accumulation of OZ439 was less than 2-fold.

The study results justify pursuing the development of OZ439 in a combination therapy for uncomplicated malaria.

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CHANGES IN *PLASMODIUM FALCIPARUM* ASEXUAL AND SEXUAL POPULATIONS IN CHILDREN WITH ACUTE INFECTIONS FOLLOWING TREATMENT WITH ARTEMISININ-BASED COMBINATION THERAPIES

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Artemisinin-based combination therapies (ACTs) may influence malaria transmission but the mechanisms of their effects have not been totally elucidated. The changes in *Plasmodium falciparum* asexual and sexual populations in the first 16 h following treatment with artemether-lumefantrine (AL) or artesunate-amodiaquine (AA) were evaluated in 435 children with acute infections. In 162 children there were significant increases in peripheral asexual parasitaemia at 1 h, and in 9 of these, a simultaneous but insignificant increase in gametocytaemia at 1 h, followed thereafter by a precipitous and significant fall in all patients suggesting mobilization into peripheral circulation before a lethal effect. A fifth of mobilized gametocytes were young with a peak at 1 h. Young gametocytes were not found in peripheral blood after 16 h. Time-course of gametocyte sex ratio (GSR) showed a female-male-female-biased cycle at 0 h, 4 h and 8 h, respectively suggesting a selective lethal or removal effect on male gametocytes. Time-course of GSR was independent of density in another cohort of 52 gametocyte carriers treated with AL or AA. All dynamic effects were similar in AL- and AA-treated children. AL or AA acutely mobilize asexual and sexual parasites and alter GSR before a lethal effect, and may reduce transmission by these mechanisms.

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CONSTRUCTION OF AN ANTIMALARIAL SET OF COMPOUNDS: A PUBLIC TOOL TO BOOST LEAD DISCOVERY

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Drug resistance to current antimalarials is widespread and no new class of antimalarials has been introduced into clinical practice since 1996. Target-based lead discovery has produced disappointing results, generally for lack of whole-cell activity of new compounds. To secure that property in all chemical starting points for new antimalarial leads, we have tested the approximately 2 million-compound library used for high throughput screening at GlaxoSmithKline for inhibitors of *Plasmodium falciparum* intraerythrocytic stages. Using a modified assay based on *P. falciparum* LDH activity as surrogate of parasite growth, we have found ca. 31K primary hits inhibiting *Plasmodium* growth more than 50% at a final compound concentration of 2 mM. To maximize the chances of selecting submicromolar inhibitors only those hits inhibiting more than 80% were retested to confirm activity (ca. 19K compounds). As result of these experiments a set of 13K compounds has been selected as confirmed inhibitors. This set has been named TCAMS (Tres Cantos Antimalarial set). Additional experiments to add knowledge to TCAMS have been performed and will be described. In order to encourage further research by the larger malaria community on the cellular targets and mode of

action of the compounds, we have made public the chemical structures, together with the above mentioned data. All the information is available at the next URL: http://www.ebi.ac.uk/chemblntd and ideally, this could catalyse a world-wide chemical genomics approach to better understand the drugable genome of *P. falciparum.*

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ADVERSE EVENT PROFILE OF SEVEN-DAY COURSES OF ARTESUNATE IN PATIENTS WITH ACUTE *FALCIPARUM* MALARIA

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In a clinical trial of artesunate monotherapy for the treatment of uncomplicated *P. falciparum* malaria in otherwise healthy adults, 143 patients were randomized to receive one of three artesunate regimens: 2, 4 or 6 mg/kg/day for 7 days (n=75, 40 and 28). Adverse events (AEs) were recorded daily for the first 7 days, then weekly for a further 5 weeks. Symptoms present at baseline were not classified as AEs unless they worsened significantly. Overall artesunate was well-tolerated; only 1 patient had to have a dose repeated. One patient in AS6 was withdrawn on Day 1 after developing signs of severe malaria. In total 375 separate AEs were recorded, of which 149 (40%) occurred during the week of artesunate treatment, 67 in AS2, 46 in AS4 and 36 in AS6. During week 1 gastrointestinal disturbances were relatively common and reported by 12%, 35% and 36% patients in AS2, AS4 and AS6 respectively; in contrast, neurological AEs (5%, 3% and 1% of patients) and rashes (3%, 3% and 5%) were relatively uncommon and did not appear to be affected by artesunate dose. Laboratory abnormalities were detected in 44, 53 and 50% patients in the 3 dosing groups; however the majority of these were due to eosinophilia, which was probably attributable to treatment of the malaria infection. The most significant laboratory abnormality was a reduction in absolute neutrophil count which was detected after 3 doses of artesunate in 2 patients (1 in AS4 and 1 in AS6), and in a further 4 patients in AS6 one week after artesunate monotherapy had been completed. This finding led to closure of the high-dose artesunate arm and early termination of the trial. In conclusion these findings confirm that artesunate remains a well-tolerated drug even at high daily doses given for 7 days. However the occurrence of neutropenia in the high-dose arm 7 days after completion of treatment indicates that artesunate has a significant myelosuppressive effect and should be used with caution when high cumulative doses are given.

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A PHASE 4 RANDOMISED STUDY TO ASSESS THE TOLERABILITY OF ARTESUNATE-AMODIAQUINE (ASAQ) AND ARTEMETHER-LUMEFANTRINE (AL) FIXED DOSE COMBINATIONS (FDCS) FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* (PF) MALARIA IN LIBERIA

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Thorough assessment of the tolerability of new artemisinin-based combination therapies (ACTs) in the post-registration phase is needed, especially for the detection of adverse events (AEs) of potential concern such as haematological, liver or neurological toxicities. Our objective was to provide information on ASAQ and AL tolerability in patients >= 6 years (a less studied population), in a highly endemic area. An open-label, randomized controlled trial was conducted in 1000 patients with uncomplicated Pf malaria in Nimba County, Northern Liberia. Drug allocation was 1:1 (ASAQ Winthrop® : AL Coartem®) to a 3-day observed oral regimen, dosed by weight (ASAQ once daily; AL twice daily with fatty food). Treatment emergent clinical signs and symptoms (open questions) and laboratory events were collected until Day28. PCR corrected cure rates were measured. Overall, 92.1% (ASAQ) and 90.2 % (AL) of patients reported at least one AE. Severe AEs were rare and mostly asymptomatic (ASAQ: 3.4%; AL: 1.6%; p=0.064). Derangement of liver function tests (ASAT/ALAT increases) classified as severe was infrequent (ASAQ: 0.6%; AL: 0.2 %). Few severe but asymptomatic neutropenia AE were reported (ASAQ: 0.4%; AL: 0.2%), and only one patient with moderate anemia at baseline developed severe anemia (ASAQ arm). Some clinical AEs, i.e. fatigue (ASAQ: 39.8%, AL: 16.3%; p<0.001), vomiting (ASAQ: 7.1%, AL: 1.6%; p<0.001), nausea (ASAQ: 3.2 %, AL: 1.0 %; p=0.015) as well as anemia (ASAQ: 14.9%, AL: 9.8%; p=0.013) were reported with significantly higher frequency in the ASAQ arm. Clinical AEs were almost exclusively mild to moderate, occurred mainly during the first 3 days and did not lead to treatment discontinuation. No significant neurological AE was reported. Day28 PCR-corrected efficacy rates were high (ASAQ: 98%, AL: 100%) (No follow-up between Day7-28). Both ASAQ and AL were well tolerated in this large >= 6years population sample, with no AE-related treatment discontinuation. Notably, hepatotoxicity, neutropenia, anemia or clinically significant neurological toxicities were of no major concern in this study. Efficacy was high with both treatments.

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A SURVEY ON CLINICAL SAFETY OF 8-AMINOQUINOLINES

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The 8-aminoquinoline (8-AQ) antimalarials are the only class effective against *Plasmodium vivax* relapse and *P. falciparum* gametocytes. It has long been known that the patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency developed hemolysis and hemolytic anemia (HA) after the administration of 8-AQs. The purpose of this review is to compile all safety data in humans to better define risk-benefit of this class in an era of malaria elimination. We collected and reviewed the unpublished and published literature on safety of 8-AQs studies in human

by contacting research experts in the field, exploring and extracting database from libraries where data are available. Papers in languages other than English were also accessed. Data were entered and analyzed in Excel (2007) and SPSS for Windows, version 16. Between 1926 and December 2009, a total of 589 clinical series of 8-AQ in humans (N=469,375) were identified by the searches. An extensive literature on the safety of 8-AQs, mainly primaquine and pamaquine in humans has been identified. Primaquine receiving patients (N=435,626; 92.8%) were the highest proportion among 8-AQs receiving patients, followed by pamaquine (N=22,735; 4.8%). Among pamaquine receiving patients, 170 (0.7%) had HA resulting in 60 patients required blood transfusion and 10 patients had discontinuation of the drug. Six patients (0.3%) in pentaquine receiving patients had HA and 5 patients had to stop the drug. In primaquine receiving patients, 304 (0.07%) had HA resulting in 25 patients required blood transfusion and 43 patients needed to stop primaquine. Two patients (2/2395; 0.08%) in tafenoquine reported HA and one required blood transfusion. In overall literature, 11 deaths had been identified with pamaquine and only 4 deaths attributable to primaquine were identified in G6PD deficient children. No deaths were reported in other 8-AQs. In conclusion, this database will help public health official make risk-benefit decisions about of the use of the class of drugs for routine malaria management and in elimination operations.

1250

A RANDOMISED, DOUBLE-BLIND PLACEBO CONTROLLED TRIAL OF TWO DIFFERENT DOSING REGIMENS OF DIHYDROARTEMISININ-PIPERAQUINE FOR INTERMITTENT PREVENTIVE TREATMENT OF ADULTS AT HIGH RISK OF MALARIA IN THAILAND

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Piperaquine is an effective antimalarial with a long elimination half life coformulated with dihydroartemisinin (DP). This ACT is efficacious, well-tolerated and has a long post treatment prophylactic effect. DP is a promising candidate for use as intermittent preventive therapy.In areas of low unstable transmission like the Thai-Myanmar border it is often young adult males who bear the brunt of malaria,as their occupation involves travel and exposure to foci of higher transmission. Between October 2006 and June 2008 a double-blind, randomised, placebo-controlled trial was performed in 1000 adult males at risk of acquiring malaria but with a negative malaria smear, attending the clinics of the Shoklo Malaria Research Unit in northwest Thailand.They were randomised into 1 of 3 groups: i) monthly DP treatment (DPm) ii) alternate month treatment (DPalt)and iii) monthly placebo. The primary endpoint was the protective efficacy at 36 weeks of follow-up, defined as one minus the rate ratio of the incidence in the treatment group compared to placebo. The incidence rate of malaria on DPm was 32 episodes per 1000 person-year at risk(PY),compared to 1068 episodes per 1000 PY in the placebo group giving a protective efficacy of 97% (95% CI 95-99, p=0.001). The incidence in the DPalt group was 239 per 1000 PY giving a protective efficacy of 78% (95% CI 72-80, p=0.001)compared to placebo. The number needed to treat(NNT)to prevent an additional case of malaria with DPm and DPalt compared to placebo would be 1 and 4.8 subjects respectively. The adverse event (AE) risk ratio in the DPm group was higher compared to the placebo group for dizziness and diarrhoea (p=0.001 for all comparisons).The mean (SD) number of episodes of AEs was 1.6 (0.5) in the DPm, 1.5 (0.4) in the DPalt, and 1.4 (0.3) in the placebo group. There was a significant difference (p<0.001) in average monthly drug concentrations for the DPm group [29.9 (29.1-30.6) mean (95% CI) ng/mL] compared to the DPalt group [15.4 (15.0-15.9) mean (95% CI) ng/mL]. The 52 subjects contracting malaria had significantly (p<0.001) lower average monthly piperaquine concentrations [11.4 ng/ml (10.3-12.4)] compared to subjects without malaria episodes (n=747) [23.1 ng/ml (22.6-23.6)]. Monthly DP treatment brought about a dramatic reduction in the incidence of clinical episodes of malaria in adults compared to placebo and may be considered as an alternative malaria control strategy in high risk groups.

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SOCIOCULTURAL FACTORS THAT INFLUENCE THE IMPLEMENTATION OF ACT INTERVENTIONS IN TANZANIA: BASELINE RESULTS FROM A QUALITATIVE EVALUATION

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In 2010 Tanzania will begin providing subsidized antimalarial combination therapy (ACT) through retail drug outlets and supplying all government health facilities with malaria rapid diagnostic tests (RDT). A multidisciplinary evaluation to assess the effectiveness of these interventions on access and targeting of ACTs is currently underway in three regions. One component is examining sociocultural factors influencing implementation in two focus districts. We are using rapid ethnographic methods to document provider and community experiences with malaria diagnosis and treatment. Data will be collected at baseline and post-intervention and timed to capture seasonal differences. Baseline data collection in one site began in November 2009 and included 7 in-depth interviews with health facility and retail drug providers about their experiences with malaria case management, 4 focus groups with community members about their care seeking behaviors and experiences with ACT, and 15 in-depth interviews with people who experienced a recent fever episode to document actual care-seeking behaviors. Artemether-Lumefantrine (ALu) was viewed in a positive light at 3 of the 4 health facilities visited but was perceived as no longer efficacious at the fourth. Although retail providers also had a positive opinion of ALu, none were legally able to sell it. Some community members spoke of ALu's ability to cure malaria or cause side effects as dependent on an individual's body or blood group rather than on the drug itself**.** Although many viewed ALu in a positive light, complaints about "too many" tablets in the treatment dose were common. Both providers and community members decried the lack of diagnostic tests in government dispensaries. Community members referred to clinical diagnosis as "treating by guessing" and voiced concerns that ACTs were being prescribed for non-malarial illnesses. Despite these concerns, community members expressed a preference for health facilities over retail outlets for malaria treatment. In conclusion, provider and community perceptions of ALu are mostly positive, although some concerns about its efficacy and treatment regimen were noted. Widespread complaints about the lack malaria diagnostics suggest that the implementation of RDTs will be positively received, although acceptance of RDT results remains to be seen.

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INVESTIGATING THE RELATIONSHIP BETWEEN PRIMAQUINE HEMOTOXICITY AND ANTI-MALARIAL EFFICACY THROUGH METABOLIC PROFILING I: METABOLITE IDENTIFICATION

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The 8-aminoquinoline drug primaquine (PQ) is currently the only approved drug for the treatment of relapsing malaria, yet PQ is known to cause hemolytic anemia in patients with G6PD deficiency. Proposed mechanisms of toxicity suggest a role for transient reactive oxygen species formed

as a byproduct of metabolism. Interestingly, evidence also points to an active metabolite playing a vital role in the drug's antimalarial efficacy. A complete understanding of the overall metabolic profile of PQ is necessary to identify potential routes to circumvent toxicity while maintaining efficacy. Our lab is currently seeking to fully characterize the role of biotransformation in both the efficacy and toxicity of PQ. Accordingly, we have initiated a series of experiments to detect previously postulated metabolites, identify and characterize previously unknown species, and determine the role of individual enzymes in PQ's metabolic pathway. We have begun to explore metabolite formation in a series of *in vitro* systems, including microsomal and hepatocyte incubations, as well as in *in vivo* samples from collaborators' pharmacokinetic studies. These initial studies indicate the presence of several previously postulated metabolites, such as carboxyprimaquine, 5-hydroxyprimaquine, and 6-desmethylprimaquine, as well as suggesting the presence of species that have not been previously reported, such as aldehyde, acetyl, and ketone metabolites. In conjunction with enzyme phenotyping studies (see "Investigating the Relationship Between Primaquine Hemotoxicity and Anti-malarial Efficacy Through Metabolic Profiling II: Enzyme Phenotyping"), we seek to better understand interspecies differences in PQ metabolism that may enhance interpretation of animal models, correlate metabolites with specific enzyme pathways, and provide insight into the relationship, if any, between PQ hemotoxicity and anti-malarial efficacy.

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INVESTIGATING THE RELATIONSHIP BETWEEN PRIMAQUINE HEMOTOXICITY AND ANTI-MALARIAL EFFICACY THROUGH METABOLIC PROFILING II: ENZYME PHENOTYPING

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The 8-aminoquinoline drug primaquine (PQ) is currently the only drug in use against the persistent malaria caused by the hypnozoite forming strains *Plasmodium vivax* and *P. ovale*. However, the widespread use of PQ is complicated by its known hemotoxicity in patients with a genetic deficiency in G6PD, an enzyme implicated in cells' ability to combat oxidative stress. To date, the exact mechanisms and metabolic species responsible for PQ's hemotoxic and anti-malarial properties are not well understood. A better understanding of the metabolic profile of PQ may enable the development of analogs that maintain efficacy while minimizing or eliminating hemotoxicity. In the present study, the metabolism of PQ was evaluated in several *in vitro* systems, including: human hepatocytes and pooled human liver microsomes, with and without enzyme inhibitors; and recombinant metabolic enzymes from the cytochrome P450 (CYP), monoamine oxidase (MAO), and flavin-containing monooxygenase (FMO) families. PQ metabolism was measured as the decline of the parent compound peak in LC-MS chromatograms. As a result of this work, CYPs 1A2, 2D6, and 3A4, as well as MAO-A, MAO-B, and FMO-3 have been implicated as the key enzymes associated with PQ metabolism. Enzyme kinetics studies are currently in progress and will be presented. The incubation mixtures were also examined as part of our metabolite identification studies (see "Investigating the Relationship Between Primaquine Hemotoxicity and Anti-malarial Efficacy Through Metabolic Profiling I: Metabolite Identification"). Correlation of metabolites with specific enzymes will allow for a more complete understanding of the pathways associated with PQ metabolism and the relationship between efficacy and toxicity.

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EVALUATION OF THE COMPARATIVE EFFICACY AND SAFETY OF ARTEMETHER-LUMEFANTRINE, ARTESUNATE PLUS AMODIAQUINE AND ARTESUNATE PLUS AMODIAQUINE PLUS CHLORPHENIRAMINE (ARTEMOCLO™) FOR ACUTE UNCOMPLICATED MALARIA IN NIGERIAN CHILDREN

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Artemisinin based combination therapy (ACT) is the current gold standard for the treatment of acute uncomplicated malaria. Amodiaquine (AQ) plus artesunate (AS) is one of the preferred ACTs. Chlorpheniramine (CP) has been shown to enhance the efficacy of amodiaquine. In an open labeled randomized trial, the comparative efficacy and safety of artemether-lumefantrine (AL), artesunate-amodiaquine (ASAQ) and artesunate-amodiaquine-chlorpheniramine (AQC) was evaluated in 159 Nigerian children aged 6months to 10years with acute uncomplicated malaria. Enrollees were randomized to receive AL, ASAQ or AQC at standard doses over 3 days. (AQC: 100mg AS + 300AQ + 4mg CP/tablet using AQ 10mg/kg/day for dosing). Assessment was by 28day WHO 2003 efficacy test (PCR unadjusted cure rates only). 144/159 (90.6%) completed the study. Mean fever and parasite clearance times for AL, ASAQ and AQC were similar (ρ=0.94 and 0.12 respectively). Day 14 ACPR was 100% for AL and AQC while that for ASAQ was 98% (ρ=0.39). Day 28 ACPR were 91.1%, 92% and 95.9% for AL, ASAQ and AQC respectively (ρ=0.62). ACPR at day 42 for 115/144 (79.9%) evaluable children were similar (ρ=0.48). AQC gave the best parasitemia clearance and hematological recovery on day2 (ρ=0.022 and 0.018 respectively). The three ACTs were well tolerated. In conclusion, the three drugs were efficacious and safe. AQC gave non-significant higher ACPR than the other two on days 28 and 42. The better hematological recovery and parasite clearance with AQC on day 2 may be a fine indication of the enhancement ASAQ effect.

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INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE IN PREVENTING MALARIA AND ANEMIA IN PREGNANCY AMONG WOMEN VISITING KORLE-BU TEACHING HOSPITAL, ACCRA, GHANA

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Malaria and anemia takes a great toll on women in sub-Saharan Africa in terms of maternal morbidity and adverse birth outcomes. Intermittent preventive treatment during pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) is currently recommended for prevention of malaria in pregnancy in endemic areas. However, the effectiveness of this approach in preventing malaria and anemia during pregnancy is unclear. The objective of the study was to evaluate the effectiveness of IPTp-SP in preventing malaria and anemia among pregnant women attending antenatal clinic (ANC) at Korle-Bu Teaching Hospital (KBTH) in Accra, Ghana. A cross-sectional study comparing malaria and anemia incidence among pregnant women using IPTp-SP with non-IPTp-SP users was conducted. A total of 363 pregnant women were recruited of which 202 were users of IPTp and 161 were IPTp non-users. Malaria parasites and hemoglobin levels were determined. Thirty-one (15.3%) women using IPTp had malaria compared to 72 (44.7%) of women who did not use IPTp, $p < 0.001$. The number of anemic women not utilizing IPTp was significantly higher (58.4%, 94/161) than women using IPTp (22.8%, 46/202), $p < 0.001$. Controlling for

age and other variables, the difference in the incidence of malaria (odds ratio (OR) = 0.26, 95% confidence interval (CI) = 0.15 - 0.44, $p < 0.001$) and anemia (OR = 0.19, 95% CI = 0.11 - 0.34, $p < 0.001$) remained significant. Regardless of reported resistance to SP for malaria treatment, the IPTp-SP regime is effective in preventing malaria and anemia among pregnant women visiting ANC at KBTH. The implementation of the IPTp-SP strategy holds great promise for reducing the burden of malaria and anemia in pregnancy in Ghana.

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COST AND COST-EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN INFANTS WITH SULFADOXINE PYRIMETHAMINE IN SENEGAL

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Intermittent Preventive Treatment in infants (IPTi) is a new promising intervention for malaria control in Sub Saharan Africa which can be delivered through the existing routine Expanded Programme on Immunization (EPI). Although the efficacy of IPTi is proven, there is limited economic evaluation data on scaling up IPTi using sulfadoxine pyrimethamine (SP) as a strategy for malaria control in Senegal. Implementation scale-up cost was calculated by estimating IPTi incremental costs in the start-up year and in subsequent years. Costs included were the financial costs of delivering IPTi to infants (drugs and equipment) and of programme activities. In recurrent years, because a survey in IPTi areas showed high acceptability and health care worker knowledge, programme costs included only IPTi administration and safety surveillance. To estimate IPTi cost-effectiveness (IPTi net cost per case of malaria averted, per death averted, per year of life saved, and per disability adjusted life years) we calculated the intervention's economic costs in recurrent years using a pooled efficacy analysis. A time and motion study was also conducted to assess staff time on IPTi delivery and the associated costs. In order to delivery IPTi-SP to 17,500 infants the total amount of the financial expenditure of programme implementation was $40,753. IPTi incremental financial costs on start up year (3.31 $US/infant) were substantially higher than in recurrent years (1.043 $US/infant). In startup years communication activities mobilized the most important expenditure (30%) meanwhile, half of the total programme resources is allocated to capacity building development of professional staff. In routine, the part of programme costs was US$ 0.81 per infant, and patient costs (drug and utensils) only 23.7 US cent/infant. The time needed to administer IPTi was an average of 2.19 min/child, representing 7% of the time spends by health workers in immunization clinics. The net cost per averted case of malaria was $22.11.The cost per death averted was $ 447, per year of life saved $ 23.84 and per DALYs $ 25.39. In conclusion, IPTi with SP through EPI can be scaled up successfully at a low cost. Using pooled efficacy results from previous IPTi trails we can see that it is a highly cost effective intervention in the study sites selected. But, the IPTi cost-effectiveness in the other areas in Senegal where malaria transmission is not high, worth assessing.

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MALARIA HIGH THROUGHPUT SCREENING ON A GLOBAL SCALE

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This poster provides a brief summary of the principles and performance of an Anti-Malarial Imaging Assay utilized to screen more than 2.5million compounds. In collaboration with MMV, chemical libraries, which are structurally highly diverse, have been sourced from pharmaceutical, biotech and academic partners world-wide. Data is presented on significant aspects of assay development and HTS performance, including assay throughput, precision, reproducibility, sensitivity, and hit-rate. In addition, retest confirmation in dose response against at least one *Plasmodium* parasite strain and the mammalian cell line, HEK293, performed to ascertain the selectivity index, is discussed. The data described, demonstrates the variation of library formats from the different sources, including library storage plate specifications/descriptions, compound concentration and volume, plate seals and in-plate control well availability. All of these aspects have to be considered when processing the plates for HTS, as well as the handling of large data sets. An overview of the compound libraries screened to date and how they performed in the Anti-Malarial Imaging Assay is presented.

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PLASMODIUM BERGHEI ANKA: SELECTION OF RESISTANCE TO PIPERAQUINE AND LUMEFANTRINE IN A MOUSE MODEL

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We have selected piperaquine (PQ) and lumefantrine (LM) resistant *Plasmodium berghei* ANKA parasite lines in mice by drug pressure. Effective doses that reduce parasitaemia by 90% (ED90) of PQ and LM against the parent line were 3.52 and 3.93 mg/kg respectively. After drug pressure (more than 27 passages), the selected parasite lines had PQ and LM resistance indexes (I90) [ED90 of resistant line/ED90 of parent line] of 68.86 and 63.55 respectively. After growing them in the absence of drug for 10 passages and cryo-preserving them at -80 °C for at least 2 months, the resistance phenotypes remained stable. Cross-resistance studies showed that the PQ-resistant line was highly resistant to LM, while the LM-resistant line remained sensitive to PQ. Thus, if the mechanism of resistance is similar in *P. berghei* and *P. falciparum*, the use of LM (as part of Coartem®) should not select for PQ resistance. The stability of these phenotypes indicates that underlying mechanisms of resistance are probably coded into the cell genome and hence form the platform for studies on mechanisms of resistance of LM and PQ in *P. berghei*.

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EFFICACY OF CHLOROQUINE OR ARTEMETHER-LUMEFANTRINE AGAINST *PLASMODIUM VIVAX* AND ARTEMETHER-LUMEFANTRINE AGAINST UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CENTRAL ETHIOPIA

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In-vivo efficacy assessments of the first-line treatments for both *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) are essential for effective case management in Ethiopia. In Ethiopia, first-line treatment is artemether-lumefantrine (AL) for Pf and chloroquine (CQ) for Pv. However, with stock-outs of chloroquine and limited laboratory confirmation of species, AL becomes the default treatment for all malaria infections. Between October-November 2009, we conducted a 42-day, three arm, open label study of AL for Pf, AL for Pv, and CQ for Pv in individuals >6 months age at two sites in Oromia Region who had documented Pf or

Pv mono-infection according to the standard WHO protocol. The primary and secondary endpoints were PCR uncorrected and corrected adequate clinical and parasitological response on days 28 and 42, respectively. Tests for drug levels, genotyping, and molecular markers of resistance for all three arms are currently in process to differentiate recrudescence from new infection. Of 4426 patients tested, those with confirmed *falciparum* malaria were enrolled and treated with AL (n=120); patients confirmed with vivax malaria were enrolled, randomized and treated with AL (n=122) or CQ (n=120). The uncorrected adequate clinical and parasitological response for Pf patients treated with AL was 99% on day 28 and 42. Eight Pf patients (7%) presented with Pv infection during follow-up and were excluded from the per protocol analysis. The cure rates for Pv patients treated with AL were 76% on day 28 and 58% on day 42; those for Pv patients treated with CQ were 91% on day 28 and 68% on day 42. There were very few day 3 positives with one case each in the Pf and Pv-CQ arms. There were no serious adverse events. Nausea/ vomiting and oral lesions were the most common adverse events. AL remains a highly effective treatment for uncomplicated *falciparum* malaria in the study setting. Both treatments of vivax malaria were complicated by high rates of recurrent parasitemia. Molecular studies may help to differentiate recrudescence from new infections, but in either case, CQ was more effective than AL in delaying the recurrent parasitemia. Effectively managing Pv relapse poses a major challenge to malaria control.

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NOVEL HIGH THROUGH-PUT MOLECULAR DIAGNOSTIC ASSAY DETECTS INCREASED *PLASMODIUM VIVAX* ANTIMALARIAL RESISTANCE IN PAPUA NEW GUINEA

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Amodiaquine or chloroquine plus sulfadoxine pyrimethamine (SP) have been the first line antimalarial treatments in Papua New Guinea (PNG) since 2000. This regime was designed for treating *Plasmodium falciparum* infections but because four species of human malaria parasites are transmitted in PNG, potential for development of drug resistance exists for each individual species. In numerous studies, *P. falciparum* resistance to 4-aminoquinolines and SP has been associated with mutations in *dhfr* (dihydrofolate reductase), *dhps* (dihydropteroate synthase), *crt* (chloroquine resistance transporter) and *mdr1* (multidrug resistance 1) genes. Orthologs of these genes and similar patterns of sequence polymorphisms have been identified in *P. vivax*. Following development of a novel molecular diagnostic multiplex strategy (Polymerase Chain Reaction - Ligase Detection Reaction- Fluorescent-Microsphere based Assay), we conducted a study to screen *P. vivax* for molecular markers of resistance in *pvdhfr*, *pvdhps* and *pvmdr1*. A total of 2381 blood samples collected from Madang and East Sepik Provinces were first evaluated for *Plasmodium* species infection status. Of these, 590 *P. vivax* infected samples were screened for single nucleotide polymorphisms (SNPs; 26 different alleles) using the multiplex drug resistance diagnostic assay. High rates of double and triple mutant parasites at *pvdhfr* codons 57-58-61 and mutant 117T were detected. We also detected a high rate of mutation at *pvdhps* codon 647 (S-->P) and at *pvmdr1* codon 976 (Y-->F). Patients from Madang were significantly more often infected with highly mutant parasites than in East Sepik Province possibly reflecting a different evolution of parasites populations ($p<0.01$, *dhfr* triple mutant allele). Genotyping methods developed here will improve our understanding of drug resistance in *P.vivax* and help monitor the effectiveness of new artemisinin-based combinations in PNG.

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MONITORING *EX VIVO* MALARIA DRUG SUSCEPTIBILITY IN SENEGAL AFTER INTRODUCTION OF ARTEMISININ-BASED COMBINATION THERAPIES

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Malaria remains an important public health issue in developing countries, despite efforts to reduce morbidity and mortality from this disease. Drug treatment with artemisinin-based combination therapy (ACT) is critical to achieving reduction of malaria burden. With increasing drug resistance, malaria drug policies changed in Senegal in 2006 and ACTs were introduced. In this study we determined the *ex vivo* drug resistance profile of ~250 field isolates from Thies, Senegal since 2007 using a non-radioactive DAPI assay to better anticipate resistance and ensure that ACTs are still effective. Testing each drug individually serves as an "early warning" system for reduced responses to either artemisinin compounds or the partner drugs before any clinical failures may be observed. Blood samples were collected from patients with clinical malaria during the three-month (September to December) transmission season in years 2007 through 2009. Blood samples containing 0.1 - 1% parasitemia were incubated with various drugs to determine IC50 values. For 2007, 2008 and 2009 there were 44, 110, and 90 samples analyzed, respectively. Parasites were found resistant (data by year: 2007, 2008 and 2009) to chloroquine (36.4%, 32%, 14%); amodiaquine (9%, 8%, 0%); pyrimethamine (52.27%, 68%, 74%); and tolerant to artemisinin (19%, 18%, 30%). In 2007 and 2009 quinine was tested with 27.7% and 4% resistance respectively and mefloquine showed 54% and 80% resistance in 2008 and 2009 respectively. Artesunate was only tested in 2009 and showed good correlation with artemisinin response. Several parasites stains demonstrated multiple drug resistance, with no evidence of cross-resistance between chloroquine and amodiaquine. Drug sensitivities were observed for all compound classes, however some parasites displayed significant levels of multi-drug resistance. With changing drug use, sensitivities to some drugs (such as chloroquine) are increasing. Decreasing responses to artemisinin suggests that parasites may become less responsive to these compounds and that careful monitoring is required. The decrease of artemisinin sensitivity and the increase of chloroquine sensitivity need to be continually monitored in order to inform drug policy makers.

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MOLECULAR SURVEILLANCE OF DRUG RESISTANCE MARKER GENES IN *PLASMODIUM VIVAX* ISOLATES CAUSING SEVERE MANIFESTATIONS FROM INDIA

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India is a major contributing country to the worldwide burden of *Plasmodium vivax* contributing to almost 70% of the total cases in South East Asia with more than 50% owing to *P. vivax*. With the recent reports of Chloroquine resistance in *P. vivax* and the parasite causing severe manifestations like Cerebral Malaria, ARDS, Acute Renal Failure, Hepatic Dysfunction, etc. (as reported previously) the studies on this parasite

has become important. Due to the lack of information on the resistance pattern of various antimalarial drugs in severe *P. vivax* population, we studied the degree of polymorphism in the drug resistance marker genes that can hypothetically lead to an indication of the state of resistance of these parasites. In *P. falciparum*, it has been suggested that the increase in morbidity and mortality is due to rise in prevalence of parasite resistance to various antimalarial drugs. But in *P. vivax*, it is not known whether it is driven by drug failure or some other changes in intrinsic parasite factors that enhance parasite multiplication and virulence. In this study, the analysis of various drug resistance marker genes (mdr-1 and crt-o for CQ resistance and dhfr and dhps for SP resistance respectively) showed mainly the wild type genotype. However, in contrast to the reported limited polymorphism in the PvDHPS gene, 4 novel mutations were observed in our severe *P. vivax* isolates. But the homology modeling and molecular docking studies showed no effect of these mutations on Sulfadoxine binding. Thus it appears to indicate the absence of a drug resistant status in these severe *P. vivax* samples. The recent reports have also suggested an increased expression of the chloroquine resistance marker genes in severe vivax malaria. The microarray expression data for our severe *P.vivax* isolates also showed any one of these genes; Pvcrt or Pvmdr-1 to be upregulated. These finding on increased expression levels of genes likely involved in antimalarials drug resistance supports to further explore potential of *these genes as* molecular markers of severe disease in *P. vivax.*

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TOWARDS VALIDATION OF GENOTYPE: RESISTANCE INDEX AS A MOLECULAR TOOL IN MALARIA DRUG RESISTANCE SURVEILLANCE IN MALI

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The resistance of *Plasmodium falciparum* to the chloroquine is conferred by point mutations on PfCRT, a protein located on the digestive vacuole membrane. PfCRT 76 T mutations was identified as a molecular marker of P *falciparum in vivo* and *in vitro* resistance to chloroquine. The prevalence of mutant allele Pfcrt 76T was used by Djimdé and collaborators in Mali to calculate the Genotype: Resistance Index. Given this importance, (GRI). We aimed in this work to enhance the use of this molecular marker in epidemiological surveillance of drug resistance in other sites in Mali. We conducted a prospective chloroquine efficacy studies in Kangaba, Kella in 2001/03 and Pongonon in 2007. Malaria is hyper-endemic in all villages with seasonal peaks of transmission. The protocol was reviewed and approved by the Institutional Review Board of the faculty of Medicine, Pharmacy and Stomatology, University of Bamako and the US national institut of Allergy and Infectious Disease. The informed consent was obtained from children parents or tutors prior to their enrollment in the study. The malaria cases were recruited according to WHO 2000 protocol. The cases were treated with chloroquine and followed on days 3, 7, and 14. P *falciparum* DNA was extracted from finger prick blood blotted onto filter paper and the Pfcrt K76T genotypes were analyzed by nested PCR. For analysis purposes, mixed infections were categorized as mutant Pfcrt 76T and GRI was calculated dividing the PfCRT 76T prevalence by the therapeutic failure rate. We included 418 cases in Kella, 336 cases in Kangaba and 259 cases in Pongonon a total of 1,013 subjects with uncomplicated malaria. We determined the IGR for children lees than 5 years, aged between 6 and 10 years and more than 11 years. We found that the parasitological failure rate was 2-3 times less than the prevalence of the mutant allele Pfcrt 76T. The IGR means were 1.2; 1.25 and 1.6 respectively in Pongonon, Kangaba and Kella in children less than 5 years. In the general population, they were 1.9; 1.7 and 2.2 respectively in Pongonon, Kella and Kangaba. Our data have shown that the prevalence of mutant allele Pfcrt 76T could be used to estimate a potential chloroquine treatment failure in Mali.

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CHLOROQUINE RESISTANCE HAPLOTYPES IN THE *PFCRT* AND *MDR1* GENES OF GHANAIAN ISOLATES USING PCR AND SEQUENCE SPECIFIC OLIGONUCLEOTIDE PROBE-ENZYME LINKED IMMUNOSORBENT ASSAY (SSOP-ELISA)

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Despite decades of research to reduce the burden of malaria disease, malaria remains a major threat to humanity causing about 1.5 to 3 million deaths a year. Prompt diagnosis and effective treatment of malaria remain the main strategies to reduce the intolerable burden of the disease in the absence of an effective vaccine. Due to the widespread resistance of *Plamodium falciparum* to the commonest and cheapest antimalarial drug, chloroquine (CQ), it is important to provide effective antimalarial drug resistance surveillance system in the country. The aim of this study was to determine the single nucleotide polymorphisms (SNPs) in the *crt,* and *mdr1* genes using PCR and SSOP-ELISA and to capture any SNPs that might have been missed by the use of PCR and restriction fragment length polymorphism (RFLP) in a previous work. The study was carried out in the Hohoe District and Navrongo War Memorial hospitals. Children <5years (male and female) reporting at the outpatients departments at the two hospitals with uncomplicated malaria were recruited. Nested PCR followed by SSOP-ELISA was performed to determine the presence of SNPs responsible for CQ resistance. Fifty-nine participants (29 males and 30 females) were recruited. The SSOP-ELISA results showed the baseline prevalence of the common CQ resistance haplotype CVIET observed for all resistant parasites to be 60% at Navrongo and 56% at Hohoe and the less common haplotype SVMNT (found sporadically in Africa) was 10% at Navrongo and 30.8% at Hohoe. The amino acid change at codon 86 for the *mdr1* genes had a prevalence of 90% at Navrongo and 95% and Hohoe. Using PCR and SSOP-ELISA, the SVMNT mutation which was missed in an earlier work was captured for the first time using only PCR and RFLP. PCR and SSOP-ELISA has a great potential to be used as a tool for general drug resistance surveillance in Ghana since it is faster, provides results comparable to those obtained in previous work and can also capture new point mutations.

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HIGH THROUGHPUT GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE *PLASMODIUM FALCIPARUM DHFR* GENE BY ASYMMETRIC PCR AND MELT-CURVE ANALYSIS

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Mutations within the *Plasmodium falciparum dhfr* gene confer resistance to sulfadoxine-pyrimethamine (SP). Single nucleotide polymorphisms (SNPs) within codons 51, 59, 108 and 164 in the *Pfdhfr* gene are associated with SP treatment failure. We developed an assay using asymmetric real-time PCR and melt-curve analysis to genotype clinical samples. Unlabeled probes specific to each SNP hybridize differentially to mutant and wild-type sequences within the amplicon, generating distinct melting curves. Analytical validation was performed using plasmids, genomic DNA from reference strains, and parasite cultures. Correct genotypes were identified with 100 copies of template. The performance of the assay was evaluated with a blind panel of clinical isolates with low parasitemias. Concordance with DNA sequencing ranged from 84% to 100%. Our assay provides a number of technical improvements that facilitate high throughput screening of patient samples to identify SP resistant malaria.

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PREVALENCE OF *PFMDR1* AMPLIFICATION IN *PLASMODIUM FALCIPARUM* FROM FIVE SITES IN CAMBODIA

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Artemisinin resistance is a potentially serious threat to current artemisinin combination therapies (ACT) for *Plasmodium falciparum* malaria. In Cambodia, resistance to artesunate-mefloquine (A+M), the current first-line therapy for uncomplicated *P. falciparum* malaria in most of the country, may pose a significant problem. Since resistance to A+M is likely to arise on a background of mefloquine resistance, we sampled parasite genomes from five sites around Cambodia and assayed for amplification of the *pfmdr1* gene, a mutation associated with mefloquine resistance. We measured *pfmdr1* copy number using a previously described quantitative RT-PCR assay using the single copy *P. falciparum* beta-tubulin gene as an internal control. Among 776 cases the preliminary estimated prevalences and 95% confidence intervals of parasites with >1.5 copies of *pfmdr1* at the sites were, Chumkiri, 0.64 (0.49, 0.77); Trapaing Prasat 0.51 (0.47, 0.55); Kratie 0.09 (0.04, 0.19); Khsim 0.27 (0.12, 0.46); and Rattanakiri, 0.09 (0.04, 0.17). The estimated means and standard deviations of the *pfmdr1* copy number at the sites were, Chumkiri, 2.8 (2.1); Trapaing Prasat, 2.0 (1.4); Kratie, 1.0 (0.5); Khsim 1.3 (0.6); Rattanakiri, 1.1 (0.7). The finding of elevated *pfmdr1* copy number in Chumkiri is expected; A+M failure rates of approximately 15% were found in Chumkiri in 2006-2007. The lack of widespread *pfmdr1* amplification in the eastern sites, Kratie, Khsim, and Rattanakiri, is consistent with the reported continued efficacy of mefloquine in eastern Cambodia. The relatively high rate of *pfmdr1* amplification at Trapaing Prasat, Oddarmeanchey Province, may represent spread of mefloquine resistance from the nearby provinces on Cambodia's western border, and may represent a threat to the current first-line ACT, A+M, in this area; an *in vivo* efficacy study is underway to evaluate this possibility.

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RECTAL AND INTRAVENOUS ARTESUNATE FOR SEVERE MALARIA: MODELLING THE IMPACT OF ARTEMISININ RESISTANCE

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Treatment of adult severe malaria with intravenous artesunate greatly reduces mortality when compared to quinine and is thus recommended first-line treatment for severe malaria in adults worldwide. A large study is underway to verify if the same is true in children. Pre-referral treatment with rectal artesunate for severe malaria was shown in a recent large study to reduce mortality in those whose treatment would be otherwise delayed. As these treatments become more widely available across the tropics there is great concern that the newly discovered artemisinin resistance in Cambodia may spread and compromise their effectiveness. A mathematical model for the spread of artemisinin resistance at the population level was developed based on data from two large community-based trials of rectal artesunate, two large hospital-based trials of intravenous artesunate and recent data on artemisinin resistance from Cambodia. Various scenarios were considered including the introduction of these therapies alone and in combination, at different levels of coverage, in a variety of transmission settings and with varying efficiency of the referral system after administration of rectal artesunate. The model was used to predict the likely impact of a policy change to these therapies on the potential spread of artemisinin resistance and explore the effects of this resistance on malaria mortality and morbidity in Africa and Asia.

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SULPHADOXINE-PYRIMETHAMINE BASED COMBINATION THERAPY IN TREATMENT OF UNCOMPLICATED MALARIA IN MALI: A RANDOMIZED CLINICAL TRIAL IN TWO VILLAGES ON CHILDREN LESS THAN FIVE YEARS

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Artemisinin-based combination therapies are now first line drugs in malaria treatment in Africa. Although they still remains effective in treatment of uncomplicated malaria in Africa, little cases of resistance rised in some areas in southern Asia. We initiated non ACT study to assess the effectiveness of SP+Amodiaquine (AQ) and SP+Artesunate (AS) vs. SP alone in two Malians villages. The purpose of this study was to assess the clinical and parasitological responses these SP combinations on uncomplicated *P. falciparum* malaria in Kolle and Bancoumana (both hyper-endemic areas). We conducted a prospective study from August to December 2004 and from July to December 2005 on children aged between 6 to 59 months. We used the *in vivo* standard follow up of 28 days of WHO for efficacy assessment. A total of 912 patients were included with 304, 306, and 302 in SP+AQ, SP+AS and SP alone respectively. We registered a total of 2% loss to follow-up. We observed only four (4) cases of early therapeutic failure (1.4%) all in the arm of SP alone. We found 1%, 1.7%, and 0% of late clinical failure (LCF) and 4.1%, 3.4% and 1.4% of late parasitological failures (LPF) in SP, SP+AS and SP+AQ respectively. We found that SP+AQ was less efficacy than SP+AS (p=0.024) and SP (p=0.043) regarding to LCF and LPF respectively. The rate of clinical and parasitological adequate response (RCPA) was 93.5%, 94.9% and 98.6% for SP, SP+AS and SP+AQ respectively. There was statistical significant differences between the three groups (p=0.007). After PCR correction using MSP2, the re-infection rates were 3.7%, 4.1%, and 1.4% in SP, SP+AS and SP+AQ group respectively. PCR-corrected cure rates at day 28 were 97.2%), 99%) 100%) in SP, SP+AS, and SP+AQ and the difference was statistically significant (0.01). In conclusion, sulphadoxine-pyrimethamine only and its combination with artesunate or amodiaquine are clinically and parasitologically effective in Mali.

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DIFFERENT MUTATION RATES IN THE DIHYDROFOLATE REDUCTASE AND DIHYDROPTEROATE SYNTHASE GENES IN *PLASMODIUM VIVAX* POPULATIONS FROM CHINA

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Plasmodium vivax genes encoding dihydrofolate reductase (*pvdhfr*) and dihydropteroate synthase (*pvdhps*) are considered to play a key role in folate biosynthesis. Point mutations in *pvdhfr/pvdhps* have been used to predict resistance to antifolates. In southern Asia and Africa, P. vivax

is considered highly resistant to sulfadoxine-pyrimethamine (SP) as an increasing use of SP in malaria treatment. In China, however, few pvdhfr/pvdhps mutants have been demonstrated until recently. We used direct sequencing to examine the prevalence of mutations in pvdhfr/pvdhps in 122 P. vivax clinical isolates collected from two areas in central (Anhui) and southern China (Guizhou). For pvdhfr, 36.9% were wild-type, whereas mutations were detected at four codons (57, 58, 61, and 117). S117N/T mutation was the most prevalent (48.4%), followed by the T61M mutation (18.9%). Six pvdhfr mutant alleles were found, ranging from 37.7 to 0.8%. The most prevalent mutant haplotype among all examined samples was F57S58T61N99S117 (36.9%). The dramatically different pvdhfr allele frequencies between the two P. vivax populations might be resulted from different drug histories or intrinsic difference between temperate and subtropical strains. In contrast, except polymorphisms within a repeat region, no resistance-conferring mutations were detected in pvdhps. Together with past clinical studies of pyrimethamine efficacy, our result suggests that P. vivax populations in China may be relatively susceptible to sulfadoxine-pyrimethamine.

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IN VITRO AMODIAQUINE RESISTANCE AND ITS ASSOCIATION WITH MUTATIONS IN PFCRT AND PFMDR1 GENES OF *PLASMODIUM FALCIPARUM* ISOLATES FROM NIGERIA

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Amodiaquine (AQ) is currently being used as a partner drug in combination with artesunate for treatment of uncomplicated malaria in most endemic countries of Africa. In the absence of molecular markers of artemisinin resistance, molecular markers of resistance to AQ can be useful for monitoring the development and spread of parasites resistance to Artesunate-Amodiaquine combination. This study was designed to explore the potential role of polymorphisms on pfcrt and pfmdr1 genes and parasite *in vitro* susceptibility for epidemiological surveillance of amodiaquine resistance in *Plasmodium falciparum*. The modified schizont inhibition assay was used to determine *in vitro* susceptibility profiles of 98 patients' isolates of *P. falciparum* to amodiaquine. Polymorphisms on parasites pfcrt and pfmdr1 genes were determined with nested PCR followed by sequencing. The geometric mean of AQ 50% inhibitory concentration (IC50) in the 98 *P. falciparum* isolates was 21.287±3.61nM (range 1.25- 183.20nM). Molecular analysis showed presence of mutant pfcrtThr76, pfmdr1Tyr86 and double mutant pfcrtThr76+pfmdr1Tyr86 alleles in 76%, 48% and 35% of the isolates respectively. The geometric mean of IC50 of *P. falciparum* isolates harbouring both wild type pfcrtLys76+pfmdr1Asn86 were observed to be reduced (4.93nM) compared to isolates harbouring double mutant pfcrtThr76+pfmdr1Tyr86 (50.29nM). Reduced *in vitro* susceptibility of *P. falciparum* to amodiaquine was significantly associated with presence of mutant pfcrtThr76, pfmdr1Tyr86 or the double mutant pfcrtThr76+pfmdr1Tyr86 (p=0.0001). Results from this study suggest that polymorphisms in pfcrt and pfmdr1 genes are important for amodiaquine resistance and therefore may be useful for epidemiological surveillance of *P. falciparum* resistance to AQ.

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ANALYSES OF *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER GENE (*PFCRT*) AND MICROSATELLITE DNA LOCI FLANKING THE GENE REVEALED GEOGRAPHICALLY DIFFERENT DISSEMINATION OF CHLOROQUINE RESISTANT MALARIA IN SOUTHEAST ASIA

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A mutation in *Plasmodium falciparum* chloroquine resistance transporter (*pfcrt*) gene in codon 76 (K76T) is associated with chloroquine (CQ) resistance and used to monitor the distribution and frequency of the CQ resistant malaria. Microsatellite (MS) DNA polymorphisms flanking the drug resistant genes can be used to study the evolution of the genes. In this study, we determined the frequency of the mutation in codon 72-76 in the *pfcrt* gene and the MS polymorphisms (2E10, 9B12, PE12A) flanking the gene, using *P. falciparum* isolates from Thailand (50 isolates), Vietnam (39 isolates) and Cambodia (26 isolates). All the isolates from Thailand were CQ resistant (CVIET). All the isolates from Cambodia were also CQ resistant (CVIET, CVIDT). In contrast, 27 of the 39 isolates (69%) from Vietnam were CQ susceptible (CVMNK), while the other 12 of them (31%) were CQ resistant (CVIET, CVIDT, CVMDT) or mixed. Expected heterozygosity (*H*: gene diversity) of the each MS locus showed that the Thai population (*H*: 0.08-0.61, average: 0.35) and the Cambodian population (*H*: 0.00-0.21, average: 0.11) were less divergent than both the Vietnamese CQ resistant population (*H*: 0.73-0.86, average: 0.79) and the CQ susceptible population (*H*: 0.37-0.97, average: 0.71). The sizes of each MS locus of the Cambodian population were identical or very close to those of the Thai population. However, the sizes of some of the MS loci of the Vietnamese population were different from those of the Thai and Cambodian populations. These results suggest that the Thai and Cambodian populations had been under strong CQ selective pressure but the Vietnamese population had not, and that the origin of CQ resistant mutation in Vietnam might be different from Thailand and Cambodia.

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MALARIA TRANSMISSION STUDIES: CAN THEY INDICATE NEW MARKERS OF DRUG RESISTANCE?

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Transmission endpoints can be included in antimalarial clinical trials, where appropriate facilities for experimental mosquito feeding exist. We have demonstrated previously that, after drug treatment, *Plasmodium falciparum* parasites carrying molecular markers of drug resistance are associated with higher oocyst burdens in mosquitoes and therefore greater transmission success. We propose that candidate molecular markers of ACT resistance should also be validated in this way, which may be possible before resistance has progressed to the level where a serious reduction in clinical efficacy occurs. We will present data from a clinical trial of

artemether-lumefantrine vs dihydroartemisinin-piperaquine in Mbita, Kenya, where more than 50 successful membrane-feeds, 7 days after drug treatment, resulted in 320 infected mosquitoes. Candidate molecular markers of ACT resistance include well-studied mutations in genes pfcrt and pfmdr1, and less well described polymorphisms in genes pfmrp, pfnhe, pfubp-1 and pfatpase6. Associations between these markers and oocyst burden in the infected mosquitoes are currently under investigation and will be presented.

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A COMPARISON OF *IN VITRO* AND MOLECULAR MARKERS OF ANTIMALARIAL DRUG RESISTANCE IN NORTHERN AND WESTERN CAMBODIA

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Decreasing artemisinin effectiveness in western Cambodian *Plasmodium falciparum* strains, characterized by elevated IC50s and increased parasite clearance times, is developing in Southeast Asia. While the focus of artemisinin resistance has been geographically limited to the Thai-Cambodian border in the Cambodian provinces of Battambang and Pailin, we are attempting to evaluate the evidence for decreasing effectiveness of artemisinins elsewhere in Cambodia. AFRIMS is currently conducting a multi-year *in vitro* parasite surveillance study in various regions of Cambodia to investigate *in vitro* and molecular markers of resistance in isolates from patients with uncomplicated *P. falciparum*. The results received during our surveillance study conducted in Anlong Veng, Oddar Meanchey province in 2009-10 (n=153) were compared with previous results obtained in our seven day artesunate mono-therapy trial in Samlot District, Battambang province in 2008-9 (n=141). Although patient enrollment data between the sites were similar, parasites from northern Cambodia appear to be more sensitive *in vitro* to artemisinins compared to those from Western Cambodia. Geomeans of *in vitro* drug sensitivity (IC50) results for DHA when compared between the two sites were significantly different by Mann-Whitney U test, with a geomean of 3.06ng/ml (Battambang) and 1.96ng/ml (Anlong Veng) respectively ($p<0.0002$). *In vitro* IC50 analysis for other antimalarial drugs; artesunate, mefloquine, quinine, chloroquine, and lumefantrine is ongoing. Comparative analysis of *in vitro* phenotype and molecular markers of parasite resistance will be presented and compared with clinical data. Despite the apparent parasite IC50 difference between provinces, the genetic basis for the apparent geographic differences in parasite susceptibility in Cambodia has yet to be determined.

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RELATIONSHIP BETWEEN CHLOROQUINE USE AND CHLOROQUINE RESISTANCE IN SUB-SAHARAN AFRICA

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Despite policy changes from chloroquine (CQ) and sulfadoxine-pyrimethamine to artemisinin-combination therapy (ACT) as the primary treatment for uncomplicated malaria in most malaria-endemic countries, these compromised drugs are still widely used. We hypothesized that differences in CQ use would be reflected in the prevalence of the molecular marker for CQ resistance, the *Plasmodium falciparum* chloroquine resistance transporter (pfcrt) polymorphism at codon 76. To characterize the extent of recent CQ use, we compared data on reported drug treatment in children from national surveys conducted between 2006 and 2007 in 21 African countries. CQ use was particularly high in West Africa, where it was the most commonly reported antimalarial in 11 of 12 countries surveyed. Reported use of CQ varied in East Africa, ranging from < 1% to nearly 50%. To investigate the effect of continued CQ pressure on drug resistance, we compared drug use data from all available national surveys (between 2000 and 2007) with temporal trends in the prevalence of CQ resistance marker pfcrt 76T from published studies in 7 African countries. The proportion of resistant genotypes stayed stable over time in three countries with sustained high CQ use (Burkina Faso, Guinea Bissau and Uganda). The prevalence of pfcrt 76T increased in Niger, the only country reporting increased CQ use during the survey period, and declined in three countries exhibiting low or sharply decreasing CQ use (Malawi, Tanzania and Kenya). These findings suggest that with declining use of CQ, we may expect to find a decline in CQ-resistant malaria. As ACT availability continues to expand in the region, CQ-susceptible malaria may begin to predominate in sub-Saharan Africa in the near- to medium-term future.

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GENETIC DISSECTION OF THE ACCELERATED ACQUISITION OF DRUG RESISTANCE IN ARMD *PLASMODIUM FALCIPARUM*

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The in-field failure of most drugs against *Plasmodium falciparum* has contributed to the major global health burden of malaria. We have previously shown that parasites from Southeast Asia harbor the Accelerated Resistance to Multiple Drugs (ARMD) phenotype, as reported previously. Parasites from South East Asia consistently acquire resistance to new and unrelated antimalarials three to four orders of magnitude more frequently than parasites from other parts of the world. By assessing the ease with which progeny from an HB3xDd2 genetic cross (as reported previously) acquire resistance to the antifolate, 1843U89, we show that the ARMD phenotype is a complex, multigenic trait, with some progeny exhibiting intermediate phenotypes. Further, we show that this increased ability to acquire resistance to 1843U89 cannot be explained by external factors such as increased survivability in the presence of 1843U89 or our experimental setup. Using QTL analysis, we have identified at least four regions on chromosomes 4, 5, 7, and 13, acting in an additive or synergistic manner to confer the ARMD phenotype. Furthermore, to validate that our previously identified loci are involved in the accelerated acquisition of multiple drugs, we report on similar recent studies using an alternative antimalarial, BMS-388891, a protein farnesyl-transferase inhibitor.

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SELECTION FOR ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM* UNDER LABORATORY CONDITIONS AND POTENTIAL MECHANISMS

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To select an artemisinin resistant line in the laboratory, we have subjected *P. falciparum* Dd2 strain to dihydroartemisinin (DHA) selection with step-wise increments of drug concentrations over 13 months. We have obtained three lines with more than 20-fold increase in IC50 DHA. However, the resistance phenotype was unstable and the parasites could regain susceptibility to DHA after three months of culture in the absence of the drug selection pressure. Phenotype analysis showed that the resistant parasite displayed cross-resistance to a number of the commonly used antimalarial drugs. Analysis of potential drug targets did not detect point mutations in *pfcrt, G7, G49, pfmrp* and *pfatp6* genes, but we identified increased copy number of *P. falciparum* multidrug resistance 1 (pfmdr1) gene associated with artemisinin resistance. In addition, the

DHA-resistant parasites also showed elevated activity of the antioxidant defense systems. Collectively, this study suggests that selection of artemisinin resistance under the laboratory conditions may be associated with multiple mechanisms

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SELECTION FOR RESISTANCE FOLLOWING A VARIETY OF ANTI-MALARIAL TREATMENT REGIMES

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Drug resistance is a serious problem in health care. In the case of malaria, resistance against most antimalarial drugs is widespread, except against the recently-deployed artemisinin derivatives. The effect of drug treatment regimens on the spread of resistance is largely unknown. Using the rodent model *Plasmodium chabaudi*, we compared the effects of a variety of 'patient' treatment regimes on infections consisting of resistant and sensitive parasites, testing the impact of each regime on host health, infectiousness and the transmission of resistant parasites. In untreated mixed infections, resistant parasites starting at low frequencies in the initial inoculation produced gametocytes at densities that were barely detectable by PCR. However, drug treatment resulted in a rapid increase of resistant parasites, causing recurrent parasitaemia, increased anaemia, and a much increased transmission potential of resistant parasites. Shorter drug courses or lower drug dosages significantly reduced the fitness of the resistant parasites without compromising host health. Conventional drug treatment aimed at radical cure resulted in the greatest fitness gain for drug resistant parasites. These results demonstrate the need for more research on the role of drug treatment regimens on the spread of drug resistance in malaria. Currently recommended regimes inadvertently impose maximal selection for resistance when resistant parasites are present in an infection. There is a need to empirically evaluate the public health consequences of treating in excess of clinical need.

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EFFECTS OF *PLASMODIUM FALCIPARUM* DIHYDROPTEROATE SYNTHASE MUTATIONS ON PARASITE FITNESS

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The antifolates sulfadoxine-pyrimethamine (SP) and trimethoprim-sulfamethoxazole (TMP/SMX) have potent activity against wild type *Plasmodium falciparum*, but activity is decreased due to resistance-mediating mutations in many areas. However, SP remains the drug of choice for intermittent preventive therapy in pregnant women and children, and TMP/SMX is widely used to prevent opportunistic infections in those with HIV infection. Resistance to antifolates is mediated by a series of mutations in the target enzymes dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), key enzymes in the folic acid biosynthetic pathway. In East Africa, parasites commonly harbor 3 DHFR mutations (S108N, N51I and C59R) and 2 DHPS mutations (A437G and K540E) that mediate an intermediate level of resistance. Additional point mutations, seen more commonly outside Africa, mediate higher-level resistance. We are studying the impact of various resistance-mediating mutations in DHFR and DHPS on the relative fitness of *P. falciparum*. To this aim, we are exploring the growth of parasites under different conditions and in competition assays. We are studying D10-strain parasites engineered to express DHPS with 1-3 mutations. In initial competition experiments, D10 wild-type and single mutant (A437G) strains outcompeted parasites with 2 (A437G + A581G or S436A + A437G) or 3 (S436A + A437G + K540E) mutations over 2 months of culture, as assessed by strain-specific PCR. Experiments to more stringently characterize relative growth of these strains utilizing folate-limiting culture conditions and quantitative PCR and to evaluate parasites with mutations in DHFR are underway. Our preliminary results suggest that resistance-mediating mutations in DHPS engender a loss of fitness compared to that of wild type strains of *P. falciparum*.

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MOLECULAR ANALYSIS REVEALED ANTIMALARIAL DRUG RESISTANCE AT THE AMAZON BASIN OF ECUADOR

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Currently, the main strategy to control malaria in Ecuador relies on rapid diagnosis and treatment. Due to therapeutic failure, the scheme using Chloroquine drug has been replaced by Artesunate for *Plasmodium falciparum* non-complicated cases of malaria. No resistance to *P. vivax* has been reported to date, though further surveillance using molecular tools could contribute to a better use of antimalarial drugs. Through SSOP-ELISA, 9 *P. falciparum* and 17 *P. vivax* blood samples from 7 communities in Sucumbíos province were analyzed for Single Nucleotide Polymorphisms in the following genes: Chloroquine resistance transporter (crt); Dihidrofolate reductase (Pfdhfr) and Dihydropteroate Synthetase (Pfdhps) for *P. falciparum* and Dihidrofolate Reductase (Pvdhfr) for *P. vivax*. Further DNA sequence analysis was used to confirm the ELISA data. The following haplotypes were identified in all *P. falciparum* samples: CVMNT at the Pfcrt gene; CNCNI at the Pfdhfr gene and the wild-type SAK at the Pfdhps gene. For *P. vivax*, 11/17 samples showed the double mutated haplotype (L2R2TS) for the Pvdhfr gene. In addition, the haplotypes FR2TN, FR1TN and FR2TS were found in 4, 1 and 1 samples respectively. A low frequency of mutations related to antimalarial drug resistance was observed in the study. The K76T mutation at the Pfcrt gene, previously reported from Colombia and Peru is dominant in the Ecuadorian Amazon basin and explains the high rate of treatment failure using Chloroquine reported in the past. Despite the high pressure to Sulfadoxyne/Pyrimethamine (S/P), low resistant haplotypes were found in Pfdhfr and Pfdhps genes. A similar scenario was found in Pvdhfr gene, which carries only double and single mutations not related with resistance to S/P. Interestingly, 4 different genotypes in Pvdhfr gene were found, suggesting a high genetic diversity of *P. vivax*. Future studies increasing our sampling size will help to better understand the distribution and implications of these preliminary findings.

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REVISITING THE ROLE OF MICROSCOPY IN *PLASMODIUM FALCIPARUM* ANTIMALARIAL DRUG EFFICACY TRIALS

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Although some *Plasmodium falciparum* antimalarial drug efficacy studies include molecular tools for the identification and discrimination of *Plasmodium* species, many of them just rely on microscopic diagnosis, as WHO recommends. Furthermore, treatment outcomes are partially assessed by the presence of post-treatment asexual parasites. The aim of this study was to determine if the sole use of microscopy in *in vivo* antimalarial drug efficacy trials might lead to the misclassification of both mono and mixed *P. falciparum* infections and an underestimation of the true number of treatment failures. A total of 250 paired blood samples (50 patients) from a 28-days *in vivo* study, conducted in Escuintla, Guatemala during 1998-1999 were analyzed. At each time-point (days 0, 3, 7, 14 and 28), blood was spotted on filter paper and a thick blood smear was prepared. Malaria diagnosis was performed through Giemsa slide examination and nested PCR of the *Plasmodium* small sub-until ribosomal gene (ssRNA). In addition to the standard methodology, quality of DNA was assessed incorporating an internal beta-globin PCR. Results show that in 87.5% of the cases, microscopy was able to correctly identify mono-infection with *P. falciparum*. However, PCR analyses show that 10.4% of the samples were mixed *P. falciparum-P. vivax* infections and 2.1% of

them were actually *P. vivax* mono-infection. Reclassification of *Plasmodium* species and treatment outcomes based on PCR results should be taken into consideration for the calculation of sample size and adjustment of end-points. Microscopy alone is not enough for the accurate identification and discrimination of *Plasmodium* species *in P. falciparum in vivo* antimalarial drug efficacy trials and the use of other techniques, such as molecular testing, are strongly recommended.

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ACTIVATION OF NADPH OXIDASE-REACTIVE OXYGEN SPECIES-INFLAMMATORY CYTOKINES PATHWAY CONTRIBUTE TO ACUTE MYOCARDIAL PATHOLOGY IN MICE INFECTED BY *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is the etiologic agent of Chagas disease. In this study, we investigated the crosstalk between *T. cruzi*-induced ROS and immune activation of pro-inflammatory response, and its role in myocardial pathology in Chagas disease. Splenocytes of infected mice, *in vitro* stimulated with *T. cruzi* antigenic lysate (TcL), exhibited a statistically significant increase in NADPH oxidase (NOX) activity, ROS production and expression level of proinflammatory and other cytokines, measured by catalytic staining, fluorometry using amplex red and H2DCFDA probes, and Bioplex-ELISA assay, respectively. Addition of apocynin (NADPH oxidase inhibitor), but not inhibitors of myeloperoxidase and xanthine oxidase, resulted in up to 98% inhibition of ROS production, and a significant decline in cytokine release (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and TNF-α) from TcL-stimulated splenocytes of infected mice. Likewise, RAW 264.7 macrophages incubated with live *T. cruzi* or TcL exhibited a substantial increase in NOX activity and cytokine (IL-1β, IL-4, IL-10, IFN-γ and TNF-α) release that was inhibited by apocynin and N-acetylcysteine (ROS scavenger) treatment. These data suggested that NOX-induced ROS signal cytokine production in macrophages and splenocytes of mice infected by *T. cruzi*. To determine the pathological significance of NOX/ROS, we treated infected mice with apocynin in drinking water. Apocynin-treated/infected mice exhibited a significant decline in endogenous and TcL-stimulated splenic cell proliferation, NOX/ROS production, and cytokines release as compared to that noted in infected/untreated mice. We observed no change in myocardial parasite burden, yet, symptoms of acute myocarditis, i.e., infiltration of inflammatory infiltrate (extensive presence of inflammatory foci or disseminated inflammation) and tissue oxidative damage (8-isoprostanes, protein carbonyls, 3-nitrotyrosine and 4-hydroxynonenal adducts) were significantly decreased in the myocardium of apocynin-treated/infected mice. We conclude that NOX-dependent ROS have an important role in regulation of splenic activation of inflammatory cells and cytokine production during acute infection, and contribute to infiltration of inflammatory infiltrate and oxidative injuries in Chagasic heart.

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DEFINING TARGET SPECIFICITY OF OXADIAZOLE COMPOUNDS ON REDOX PATHWAY MEMBERS OF THE HOOKWORM *ANCYLOSTOMA CEYLANICUM*

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Hookworms, parasitic nematodes that infect approximately 700 million people, are a major cause of anemia, malnutrition, and perinatal mortality in the tropic and subtropic regions of the developing world. Within an appropriate host, larval hookworms develop into blooding feeding adults, attaching to and lacerating the mucosa of the small intestine. Degradation of the ensuing bloodmeal leads to the production of reactive oxygen species (ROS), potentially hindering parasite development and survival. We hypothesize that adult hookworms possess fully functional thioredoxin (Trx) and glutathione (GSH) redox pathways that are essential for the breakdown of ROS produced during blood feeding and that neutralization of these redox systems will lead to reduction in hookworm induced pathogenesis. To characterize these pathways in the human hookworm *Ancylostoma ceylanicum,* we mined the NCBI *A. ceylanicum* expressed sequence tag (EST) database to identify sequences encoding representative members of the Trx and GSH pathways. Several full-length cDNAs were PCR amplified and expressed as recombinant proteins in *E. coli*. Utilizing a kinetic NADPH consumption assay, we determined the specific activity of a novel hookworm peroxiredoxin (AcePrx) and glutathione peroxidase (AceGPx). We also tested the effect of oxadiazoles, nitric oxide-donating compounds, on *A. ceylanicum* adult worms using an *ex vivo* survival assay. Exposure of adult *A. ceylanicum* to micromolar concentrations of select oxadiazole compounds resulted in the significant reduction in worm survival compared to controls treated with equivalent concentrations of albendazole or carrier alone. Furthermore, treatment of *A. ceylanicum*-infected hamsters with furoxan significantly diminished hookworm-induced anemia and worm burden in the host small intestine. This data suggests that oxadiazole compounds represent new lead drugs for the treatment of hookworm disease. Future experiments will investigate the impact of oxadiazoles on *A. ceylanicum* survival by determining the enzymatic activities targeted by these compounds in hookworms and their anthelminthic activity *in vivo*.

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METHIONYL-TRNA SYNTHETASE AS A DRUG TARGET FOR *TRYPANOSOMA BRUCEI*

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Aminoacyl-tRNA synthetases (aaRS) covalently attach amino acids to their corresponding tRNAs, and are required for protein translation. AaRS enzymes make excellent drug targets for antimicrobial drug development as evidenced by mupirocin (a topical antibiotic acting on the isoleucyl-tRNA synthetase) and REP8839 (a clinical Phase II antibiotic acting on the methionyl-tRNA synthetase [MetRS]). Sequence analysis of aaRS enzymes of trypanosomatid parasites indicates significant differences in active site residues between *Trypanosoma brucei* and human homologs of MetRS justifying additional effort to exploit this protein as a drug target. The MetRS has been subjected to RNAi experiments in bloodstream form *T. brucei* and confirm the essentiality of this protein. An aminoacylation assay has been developed to measure enzyme activity and inhibitory action of test compounds. A series of compounds related to REP8839 were synthesized and the most potent had an IC50 ~1 nM on recombinant *T. brucei* metRS. When tested on bloodstream form T. brucei, the EC50 of the most potent compound was 4 nM. Another series of compounds (pyrimidine derivatives) had EC50 values as low as 75 nM on *T. brucei* cultures. The compounds are non-toxic to mammalian cells at concentrations >20 µM. Our best compound (#1312) dramatically suppresses parasitemia in the murine model of acute *T. brucei* infection. The compounds appear to have poor penetration through the blood brain barrier as indicated by the MDR1-MDCK cell permeability assay. Thus, we are making analogs to try to improve this characteristic so as to make the compounds amenable for treating late stage *T. brucei* infection. Attempts to solve the crystal structure of the *T. brucei* met-RS bound to our inhibitors are underway so as to help guide rational drug development.

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A NOVEL FUNCTION OF THE *BRUGIA MALAYI* CATHEPSIN-LIKE CYSTEINE PROTEASES IN THE ENDOSYMBIOTIC INTERACTION WITH *WOLBACHIA*

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Wolbachia is essential for the development and reproduction of *B. malayi*, the causative agent of Lymphatic Filariasis, which opened up a search for novel anti-*Wolbachia* drug targets in addition to antibiotics. Our study was aimed at identifying proteins that potentially have an essential function in this endosymbiotic relationship. We first characterized the effects of tetracycline treatment on the regulation of *Brugia malayi* transcripts 7 and 14 days post treatment using a whole genome microarray studies. We observe primarily up-regulation of *B. malayi* transcripts encoding proteins and enzymes involved in amino acid synthesis and protein translation. Moreover, a bimodal regulation pattern of *B. malayi* transcripts encoding signaling genes and cysteine proteases was observed when tested further by *in vitro* treatment with tetracycline and qRT-PCR. This pattern may be representative of the worms' response to *Wolbachia* death in different tissues; earlier effect on embryogenesis and a later effect on the *Wolbachia* within the hypodermis of the adult worms. Filarial cysteine proteases are known to be involved in molting, embryogenesis and tissue remodeling, processes shown to be dependant on *Wolbachia*. To further elucidate the role cysteine proteases play during this symbiosis, we studied their time dependent expression pattern after anti-*Wolbachia* treatment. For example, the transcripts corresponding to *Bm-cpl-3* and *Bm-cpl-6* were found to be up-regulated 1 day post treatment, unchanged or down-regulated by day 3, but then up-regulated at day 6. Notably, RNAi knockdown of *Bm-cpl-5* that is detrimental for embryogenesis resulted in a specific reduction of *Wolbachia* in the hypodermis and microfilariae but not in the oocytes and embryos. This effect was further established by showing that in the *Bm-cpl-5* dsRNA RNAi treated worms, the transcript levels of a *Wolbachia*-specific ankyrin gene (*wBM0287*) were down-regulated by 10.2 fold. The possible role cysteine proteases play in the relationship between the endosymbiont and its *B. malayi* host will be further discussed.

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STAGE-SPECIFIC PATHWAYS OF *LEISHMANIA* ENTRY INTO MACROPHAGES

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Leishmania spp. have a life cycle with two stages: the promastigote, found in the sand fly vector and the amastigote, found in macrophages. We showed that infection of susceptible BALB/c mice macrophages with *L. infantum chagasi* increased the expression of caveolae components. Caveolae, a subset of lipid rafts, are membrane microdomains enriched in ganglioside-M1 and cholesterol. Promastigotes co-localized with the caveolae markers caveolin-1 and GM1 during entry and up to 24h after phagocytosis. Transient depletion of macrophage membrane cholesterol by 1h exposure to methyl-β-cyclodextrin (MβCD), impaired the phagocytosis of virulent, but not attenuated promastigotes. In addition, virulent promastigotes experienced increased lysosome fusion ($P<0.001$), and impaired replication ($P<0.05$), even though macrophage cholesterol was replenished by 4h. Amastigotes reside in phagolysosomes, hence we hypothesized that the use of cholesterol-rich microdomains to delay lysosome fusion is promastigote-specific. Accordingly, the entry of promastigotes, but not of amastigotes, is decreased in MβCD-treated macrophages ($P<0.001$). Furthermore, for up to 48h, the intracellular survival of amastigotes was not affected by transient cholesterol depletion. Unexpectedly, by 72h, amastigotes in pre-treated macrophages were unable to replicate ($P<0.05$). By 1h of infection, the early lysosome marker LAMP-1 was recruited to 80% and 20% of amastigote and promastigote compartments, respectively. The promastigote-to-amastigote conversion takes 24 to 48h, consequently, by 24h, the recruitment of LAMP-1 in promastigote-infected macrophages increased to 46% ($P< 0.001$). Disruption of macrophage lipid rafts increases LAMP-1 recruitment to promastigote compartments ($P<0.001$). In contrast, amastigotes readily associated with LAMP-1, regardless of treatment. Our results support the hypothesis that: virulent promastigotes exploit a caveolae-mediated pathway to enter macrophages, and this cholesterol-rich route facilitates their survival by delaying lysosome fusion until their conversion into amastigotes.

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ANTENATAL INFECTION WITH LYMPHATIC FILARIASIS INCREASES SUSCEPTIBILITY TO MALARIA IN KENYAN CHILDREN

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We previously observed that antenatal exposure to schistosomiasis in women co-infected with malaria correlated with increased susceptibility to malaria during childhood. To examine the impact of lymphatic filariasis (LF) on malaria susceptibility we undertook a prospective cohort study of 700 newborns in a malaria endemic region of Kenya in which children were examined biannually from birth to age 3 for *Plasmodium falciparum* infection and the presence of malaria antigen-specific T cell responses. 13.6% of pregnant women were co-infected with LF and malaria and 32.6% were infected with LF alone. The risk of malaria increased by 58% (OR 1.58 [95% CI 1.05-2.38] P=0.027) in offspring of women infected with just malaria while the risk increased to almost 3-fold (OR 2.87 [95% CI 1.83-4.52] P=<0.0001) in offspring of women co-infected with malaria and LF as compared to offspring of women lacking any infection during pregnancy. We hypothesized that prenatal LF exposure impairs fetal immune responses to malaria antigens *in utero* thus acquiring a more tolerant phenotype. Cord blood mononuclear cells (CBMC) of newborns of women co-infected with LF and malaria had almost no malaria antigen-driven IFN-γ/IL-2 or IL-5/IL-13 (1 and 5% respectively) compared to malaria-specific responses in CBMC from women infected with malaria alone (24 and 25%, P=0.04). This impaired T cell response persisted into childhood. A greater proportion of CBMC had malaria-antigen-driven IL-10 from women with LF and malaria as compared to women with malaria alone (P=0.05). Co-infection with LF and malaria during pregnancy was associated with reduced ability to generate MSP1-specific IgG in newborns. Thus, filariasis and malaria co-infections during pregnancy enhance risk for malaria infection in their offspring, possibly through a mechanism of immune suppression to protective malaria blood stage antigens. Treatment of filariasis and other helminths in pregnant woman may reduce the risk of malaria for their children.

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THE IMPACT OF SCHISTOSOMIASIS AND MALARIA ON THE PATHOLOGY OF DISEASE AND THE IMMUNE RESPONSE IN NON-HUMAN PRIMATE MODELS

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Schistosomiasis and malaria are the two leading parasitic diseases worldwide. These diseases cause significant morbidity and mortality,

with malaria causing over 500 million clinical cases and up to a million deaths annually. Schistosomiasis affects an estimated 200 million people and causes approximately 280,000 deaths annually. Areas endemic for schistosomiasis and malaria overlap in sub-Saharan Africa as well as other parts of the world. Studies that have examined concurrent infections in mouse models have shown that the presence of helminth infections can adversely affect the protective immune response to malaria but similar studies in humans have been less clear. We hypothesized that in concurrently infected animals, a pre-existing schistosome infection would exacerbate a subsequent malaria infection. We percutaneously exposed four rhesus macaques to 500 cercariae of *Schistosomiasis mansoni*. At eight weeks of infection, these animals plus four additional macaques were exposed to the bites of *Anopheles dirus* mosquitoes infected with *Plasmodium coatneyi*. At day 12 and 13 following sporozoite challenge, parasitemia became patent and rapidly increased in both schistosomiasis positive and schistosomiasis negative animals. Both groups of animals were treated with anti-malarial drugs when parasitemia reached high levels around days 17, 18, or 19. In the presence of anti-malarial drug treatment, schistosomiasis positive animals obtained higher parasitemia levels for a longer time period than their malaria-only infected counterparts (p<0.0001). The mechanism underlying this difference in parasitemia is presumably due to a difference in the immune response. The cytokine and antibody responses are being studied to determine specific immune responses of the two groups of animals. These studies are critical for understanding the immunological and pathological consequences of concurrent infections and can aid in the design of vaccines, drug targets, and treatment regimens for individuals living in co-endemic areas.

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ANTIGEN-SPECIFIC B MEMORY CELL RESPONSES TO *PLASMODIUM FALCIPARUM* BLOOD STAGE MALARIA ANTIGENS AND SCHISTOSOMAL ANTIGENS IN MALIAN CHILDREN WITH AND WITHOUT *SCHISTOSOMA HAEMATOBIUM*

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Polyparasitism is common in the developing world. We have previously demonstrated that schistosomiasis-positive (SP) Malian children, aged 4-8 years, have protection from malaria compared to matched schistosomiasis-negative (SN) children. Evidence of durable immunologic memory to malaria antigens is conflicting, particularly in young children, and the effect, if any, of concomitant schistosomiasis on acquisition of memory is unknown. We examined antigen-specific B memory (BM) cell frequencies (expressed as percentage of total numbers of IgG-secreting cells) in expanded peripheral blood mononuclear cells (PBMC) from Malian children aged 4-14 to malaria blood-stage antigens, apical membrane antigen 1 (AMA1) and merozoite surface protein 1 (MSP1) and to schistosomal antigens, soluble worm antigenic preparation (SWAP) and schistosoma egg antigen (SEA) during the malaria transmission season and again in the dry season. A ratio of greater than 0.01% was considered a positive response. Antigen-specific BM responses were detected in all age groups to all antigens. Memory B cell responses to SWAP, in SP children, were lower during the malaria transmission season compared to the subsequent dry season [4/16 (25%) vs. 10/16 (63%) responders, P= 0.04, X2 analysis]. Negligible SEA or SWAP-specific BM responses were detected in SN children. Enhanced MSP1-specific BM responses were noted across all age groups during the transmission season in SP versus SN children (10/16 [63%] vs. 4/16 [25%] responders, P= 0.04, X2 analysis; ratio 0.056 vs. 0.017, P=0.04) but equalized by the following dry season. AMA1-specific BM responses were greater in SP versus SN children, aged 9-14 years, during the malaria transmission season (6/8 [75%] vs. 2/8 [25%] responders, P=0.04, X2 analysis) and remained elevated at dry season follow-up (8/8 [100%] vs. 4/8 [50%], P=0.04). We conclude that detectable BM responses are present against both malaria and schistosomal antigens and that the presence of *S. haematobium* may be associated with enhanced BM responses to malaria antigens.

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MSP-1-SPECIFIC MEMORY B CELL RESPONSES ARE STABLE BUT B CELL PHENOTYPE FREQUENCIES CHANGES AFTER PROLONGED ABSENCE OF MALARIA TRANSMISSION IN HIGHLAND WESTERN KENYA

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Reduction of malaria transmission could lead to decreased B cell stimulation and lack of induction of B cell memory to *Plasmodium falciparum* antigens. Conversely, a decrease in malaria transmission might reduce the frequency of "exhausted" memory B cells seen in populations with high levels of malaria transmission. To investigate changes in B cell immunity in the absence of malaria transmission, we assessed memory B cell responses in adults living in a highland area of Kenya that recently reported interruption in malaria transmission. Proportions and frequencies of MSP1-specific memory B cells and B cell phenotypes were measured in a cohort of 57 adults at a time after malaria interruption for a one year period (April 2008) and one year subsequently (April 2009). Antigen-specific memory B cells were assessed by B cell ELISPOT and B cell phenotyping by flow cytometry. During the one-year period, none of the individuals tested developed clinical malaria. MSP1-specific memory B cell responses were present in 55% and 60% of individuals respectively in 2008 and 2009. 75% of the participants who had MSP1-specific memory B cells in 2008 maintained the responses one year later. Proportions of MSP1-specific memory B cells increased over the one year period (p=0.02). B cell phenotyping demonstrated that at the second time point, the proportion of circulating CD19+ cells increased (p=0.0001) but the proportions of activated classical memory B cells (CD19+IgD-CD27+CD21-) (p=0.0001) and splenic marginal zone B cells (CD19+IgD+IgM+CD27+) decreased (p=0.0001). The proportion of atypical memory B cells (CD19+IgD-CD27-CD21-) and classical memory B cells remained the same across the two time points. The percentages of "exhausted" memory B cells (CD19+CD19+IgD-CD27-CD21-FcRL4+) were however very low at both time points (<5%). In conclusion, MSP-1 specific memory B cells persist in adults in areas of unstable transmission, even in the absence of transmission. However, the effects of a reduction in malaria transmission on B cell immunity and phenotype may not be restricted to malaria antigen-specific responses.

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ASSOCIATION BETWEEN *PLASMODIUM FALCIPARUM* ANTIBODY RESPONSES AND AMODIAQUINE-SULFADOXINE-PYRIMETHAMINE TREATMENT FAILURE IN KAMPALA, UGANDA

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Elimination of *Plasmodium falciparum* after partially effective therapy is influenced by host immunity. We previously showed that responses

to treatment for uncomplicated malaria with amodiaquine-sulfadoxine-pyrimethamine (AQ+SP) were associated with surrogates of immunity, including age and proximity to a mosquito breeding site. To further assess associations between immunity and treatment response we studied humoral antimalarial responses in children in Kampala aged 1-10 years who received AQ+SP for treatment of uncomplicated malaria. We measured IgG responses to the following 8 *P. falciparum* antigens via ELISA in 207 pairs of serum samples collected on the day of therapy (Day 0) and 14 days after treatment (Day 14): circumsporozoite protein (CSP), liver stage antigen 1 (LSA1), apical membrane antigen 1 (AMA1), merozoite surface proteins 1, 2, and 3 (MSP 1, 2, 3), and the R0 and R2 domains of glutamine rich protein (GLURP). Results were standardized against pooled immune serum from adults living in Kampala. Our primary outcome was the genotype-adjusted risk of recrudescence within 63 days. Associations were estimated using generalized estimating equations. Age-adjusted IgG responses to AMA1 on Day 0 and Day 14 were significantly higher in those living closer to the breeding site ($p<0.02$). Overall risk of treatment failure was 12%. After adjusting for age and parasite polymorphisms associated with treatment failure, the risk of failing therapy was significantly lower in those with higher AMA1 responses on Day 0 (OR=0.79 / doubling of titer, $p=0.01$). IgG responses for the other antigens were not significantly associated with treatment response, however there was a trend for protection with higher Day 0 responses to MSP 2 (OR 0.79, $p=0.06$) and 3 (OR 0.81, $p=0.09$). Our findings demonstrate that antibody responses to AMA1 are associated with blood-stage immunity as measured by host clearance of parasites in the setting of partially effective therapy.

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EVALUATION OF *PLASMODIUM FALCIPARUM* MULTI-ANTIGEN ANTIBODY DYNAMICS IN INDIVIDUALS EXPERIENCING SUCCESSIVE ANNUAL INFECTIONS LIVING IN THE HYPOENDEMIC PERUVIAN AMAZON

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Years of exposure to *P. falciparum* are necessary for the development of immunity in high transmission areas, suggesting that protective humoral responses are disabled by parasite hyperexposure. However, in low transmission areas, such as the Peruvian Amazon, malaria-exposed individuals produce immune responses leading to clinical protection after only a few infections. To investigate the antigens responsible for these effective immune responses, this study used antigen-conjugated beads in a LUMINEX system to compare the pre-, during, and post-infection antibody responses to 8 antigens, including AMA1, CSP, EBA175, LSA1, MSP1, MSP2, MSP3, and MSP6. 34 adults and 21 children with samples from 2-3 successive infections spaced by ~1 year were evaluated. We found that adults maintained AMA1, EBA175, MSP1 and MSP3 responses for >300 days post-infection, while in children responses to only MSP1 and MSP3 lasted for >300 days. Although differences in the magnitude/longevity of responses among 1st, 2nd, and 3rd detected infections are recognizable, in adults there were no significant differences for any antigen. However, antibody levels to EBA175 and MSP-3 were significantly higher among children at the 3rd detected infection than at the 1st, suggesting that these responses are boosted earlier at each subsequent infection. After correlating each antigen response to the others, some antigen pairs were associated with more parasite exposure (AMA1&EBA175, AMA1&MSP3 and EBA175&MSP2), and some were associated with less exposure (EBA175&MSP1 and AMA1&MSP1). When comparing responses in asymptomatic versus symptomatic adults, responses to MSP1, MSP2, MSP3, and MSP6 were found to be significantly higher in asymptomatics than in symptomatics at the 2nd detected infection but not at the 1st. In summary, although MSP1 produced the largest post-infection responses even at the 1st detected infection, other blood-stage antigens, particularly MSP3 (to which even children elicited long-lived responses), were found to be important players in the anti-malarial immune response despite low transmission exposure.

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HAPLOTYPES OF FC GAMMA (FCΓ) RECEPTOR (FCΓRIIA AND FCΓRIIIB) PREDICT SUSCEPTIBILITY TO HIGH-DENSITY PARASITEMIA AND FUNCTIONAL CHANGES IN CIRCULATING IFN-Γ LEVELS IN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA IN WESTERN KENYA

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The development of protective immunity against *Plasmodium falciparum* is partially mediated through binding of malaria-specific IgG to Fc gamma (γ) receptors. Human FcγRIIA-H/R-131 and FcγRIIIB-NA1/NA2 exhibit polymorphic variability associated with differential binding to IgG subtypes and malaria disease outcomes. The role of FcγRIIA-H/R131 and FcγRIIIB-NA1/NA2 haplotypes in conditioning susceptibility to high-density parasitemia (HDP; ≥10,000 parasites/μL), a clinical manifestation of severe malaria in *P. falciparum* holoendemic areas, however, is largely undefined. As such, the role of FcγRIIA-H131R/FcγRIIIB-NA1/NA2 haplotypes was investigated in children (n=528) presenting with acute malaria at a rural hospital in western Kenya. Since variations in the FcγR may alter interferon gamma (IFN-γ) levels, a mediator of both innate and adaptive immune responses, additional functional analyses were carried out in the context of the FcγR haplotypes. Results reveal that circulating IFN-γ was negatively correlated with parasitemia levels (r=-1.740, P=0.005). Children with HDP also had lower circulating IFN-γ levels than the non-HDP group (P<0.001). Multivariate logistic regression analyses controlling for covariates revealed that carriage of the FcγRIIA-131R/FcγRIIIB-NA1 haplotype was associated with protection against HDP (OR; 0.48, 95%CI, 0.31-0.76; P=0.002), while carriage of FcγRIIA-131H/FcγRIIIB-NA1 haplotype increased susceptibility to HDP (OR; 1.49, 95%CI, 1.04-2.14; P=0.031) relative to individuals without these haplotypes. Carriers of the FcγRIIA-131H/FcγRIIIB-NA1 (131H/NA1) haplotype had significantly lower IFN-γ levels relative to non-carriers (P=0.046), while FcγRIIA-131R/FcγRIIIB-NA1 (131R/NA1) haplotype had elevated IFN-γ levels relative to non-carriers (P=0.067). These results demonstrate that variations at the FcγR gene are associated with functional changes in IFN-γ production, and susceptibility to HDP in children with *falciparum* malaria.

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SPATIAL DISTRIBUTION, HABITAT CHARACTERIZATION AND DYNAMICS OF *ANOPHELES GAMBIAE* MOLECULAR FORMS LARVAL BIOTOPES ALONG AN URBANIZATION GRADIENT IN THE CITY OF YAOUNDÉ, CAMEROON

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Increasing urbanization in Africa is drawing the attention of public health managers on urban malaria, and it raises the question of whether malaria vectors have the potential to adapt to the environmental stressors normally encountered in the most densely populated cities. In the forest domain of southern Cameroon, the molecular forms M and S of *Anopheles gambiae* segregate along urbanization gradients, suggesting that a

process of adaptation by the M form to the urban environment is under way (Kamdem et al., submitted). This process is presumably driven by the ability of M larvae to develop successfully in polluted urban habitats. To characterize the larval biotopes of M and S and their dynamics, we conducted a longitudinal survey of *An. gambiae* larval habitats to assess their distribution and relationship with human activities in the capital Yaoundé and peri-urban neighborhoods. A total of 2,449 potential mosquito breeding sites were examined, of which about 20% contained *An. gambiae* larvae. Anopheline larval habitats were more abundant in urban compared to rural or suburban areas. Seasonal fluctuations in breeding sites availability were more pronounced in the rural than urban habitat. Draining streams and swamps were associated with no or very low larval densities. Human activities such as vegetable market gardening, housing in swampy areas, and construction sites were associated with breeding sites of *An. gambiae*. Unexpectedly, *An. gambiae* larvae were collected from urban breeding sites highly polluted with organic matter. PCR identification revealed that only the M molecular form of *An. gambiae* was present in the most urbanized settings, whereas the S form was by far the most abundant in the rural sites, the suburban ones being transitional between these extremes. These findings provide evidence that the malaria vector *An. gambiae* s.s. is adapting to urban waste waters, and clearly partition the distribution of the molecular forms M and S between urban and rural areas.

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SPATIAL-TEMPORAL DISTRIBUTION OF IMMATURE AND ADULT MALARIA VECTORS IN FOUR ECOLOGICAL SETTINGS IN COASTAL KENYA

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The on-going malaria control activities in Kenya through use of Insecticide treated nets (ITN) and Indoor residual spraying (IRS) necessitate up-to-date information on malaria vectors. An ecological study of the spatial-temporal distribution of immature and adult malaria vectors was conducted in eight villages (two in each of four ecological settings) in the south coast of Kenya. Longitudinal larval surveys were conducted monthly in selected larval habitats from May 2009 to March 2010 using standard dipper. Additionally, adult malaria vectors were concurrently collected using pythremum spray collection (PSC) and clay pots in 10 houses from March 2009 to March 2010. A total of 285 Anopheline larvae were sampled during the 10 months of larval sampling. The number and quality of larval habitats sampled in each ecological setting fluctuated with rainfall. *Anopheles* larvae were found most frequently in larval habitats located in the estuarine habitats, accounting for 81% of the total larvae sampled. The majority of the larvae were *An. gambiae* s.l (88%), with *An. funestus* comprising the rest (12%). Mean density of *Anopheles* larvae was 3 times higher in the estuarine habitats compared to the other three ecological settings combined. Abundance and density of *Anopheles* larvae was highly associated with depth, pH and conductivity of aquatic habitats. Correspondingly, 69% (962/1386) of the adult mosquitoes were *An. gambiae* s.l with *An. funestus* comprising the remaining 31% (424/1386). An average of 7 *An. gambiae* s.l and 2 *An. fune*stus mosquitoes were collected each month in the estuarine environment compared to <1 mosquito of each species in the other ecological settings. Overall, densities of adult malaria vectors were low throughout the study period, and were highly dependent on rainfall throughout the 12 months. Both abundance and composition of malaria vectors was dependent on the ecological setting and modulated by rainfall. While these findings are not surprising, only limited data was available for the south coast of Kenya. The findings of study will be useful for the planning and implementation of control strategies for malaria vectors.

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ENVIRONMENTAL CHANGE AND THE MICROBIAL ECOLOGY OF *ANOPHELES GAMBIAE*

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Recent studies suggest that land use changes, such as deforestation, strongly enhance the productivity of malaria vectors, and thus malaria transmission. This is because deforestation exposes aquatic habitats to sunlight, resulting in increased temperatures. Further, sunlight may induce changes in the microbial communities that mosquito larvae use for nutrition. This study utilized field-based microcosm approaches in combination with water chemistry analyses and pyrosequencing for microbial diversity in order to examine the impacts of environmental change in *An. gambiae* larval habitats and habitat vector productivity. Results of habitat productivity in different land use scenarios have demonstrated a significant effect of land use and canopy cover on larval malaria vector survivorship and habitat productivity. Survivorship in semi-forested and naturally forested areas was reduced 20% and 99%, respectively when compared to areas that had been deforested. Interestingly, when microcosm temperature in the field was controlled, temperature was shown to have the strongest effect on pupation rate while larval survivorship was more affected by algal biomass. Microbial diversity analyses show significant differences in bacterial communities from deforested, semi-forested, and forested habitats. Bacterial communities in the surface microlayers and larval guts from the same habitat also showed significant differences in composition and suggested a selective assimilation of photosynthetic microbes. These preliminary results suggest that *An. gambiae* ss preferentially feeds on photosynthetic microbes in the surface microlayer of their habitats and that the role of microbial community changes induced by light may play a more important role than temperature in some scenarios.

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PROFILING GUT MICROBIOTA IN MOSQUITO *ANOPHELES GAMBIAE* USING PYROSEQUENCING

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Mosquito *Anopheles gambiae* is a major malaria vector. Vector competence is determined by the tripartite eco-interactions among microbes, malaria and mosquito immunity. Mosquito gut harbors diverse microbial communities. However, little is known about the dynamics of the gut microbiota from larva to adult and its impact on the gut eco-symbiotic interplays. Early studies of the gut bacteria relied on culture-and/or cloning-based low throughput techniques that characterize only a small fraction of the microbiota. Using pyrosequencing approach targeting the V1-3 hypervariable region of the 16S rRNA gene, we gained an unprecedented view of the diversity present in the gut microbiota and were able to detail the dynamics of the gut microbial community from larva to adult and assessed the effect of blood feeding on the gut community. Pyrosequencing yielded 79592 sequences from 14 samples of a lab reared *An. gambiae*. The sequences correspond to 260 genera belonging to 11 phyla with dominances of *Proteobacteria*, *Bacteroidetes, Actinobacteria* and *Firmicutes*. The structure of gut microbiota changes along the life stages. Among the tags obtained from larva, more than half (54.3%) belong to the Family *Enterobacteriaceae* (unable to classify to genus). *Microbacterium* (23.7%) is abundant only in larva. Pupa

harbors a complex community with the dominances of *Elizabethkingia* (26.1%), *Acidovorax* (10.5%) and *Enterobacteriaceae* (10.8%). In newly emerged adults, flora composition becomes simpler, dominated by *Elizabethkingia* (55.9%), *Staphylococcus* (8%), *Enterobacteriaceae* (7.5%) and *Leucobacter* (6.9%). At least 7 out 20 (35%) genera that were present in pupa did not rise in the adult, likely being cleaned up by the sterilization process during the metamorphosis. *Finegoldia, Serretia* and *Pantoea* seem to be newly established in the adult. Bloodmeal reduces the diversity of gut microbiota. After blood feeding, 6 out 17 (35.3%) genera disappeared. *Elizabethkingia* and *Enterobacteriaceae* dominate the community, accounting for up to 77% of the total tags. The diversity of gut communities reduced with operational taxonomic unit (OUT) dropping from 110 to 36. Richness of the communities was estimated by ACE and Chao implemented by the software Mothur. By picturing gut communities we have gone one step further towards a better understanding the mosquito gut ecosystem.

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SYMPATRIC *PLASMODIUM FALCIPARUM - ANOPHELES GAMBIAE* POPULATIONS PRODUCE LOWER INFECTION INTENSITIES IN AFRICA

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Both *Plasmodium falciparum* and *Anopheles gambiae* show great diversity in Africa, in their own genetic makeup and malaria infection phenotypes. The genetics of the individual mosquito and parasite are known to play a role in determining the outcome of infection, but whether differences in infection phenotype vary between populations remains to be investigated. Here we conducted experimental infections using two recently established *An. gambiae* colonies from Cameroon and Burkina Faso and wild *P. falciparum* corresponding to their sympatric and allopatric populations. Infection phenotype was determined in terms of oocyst prevalence and intensity for at least nine infections for each vector-parasite combination and compared between infection types. We show that the mosquito colony used (sympatric or allopatric to the parasite) has no significant effect on infection prevalence, however has strong effects on infection intensity. Sympatric infections produced 25% fewer oocysts per midgut than allopatric infections, while prevalence was not affected by sympatric/allopatric interactions. The reduction in oocyst numbers in sympatric couples may benefit both the vector and parasite. It has been proposed that increasing the number of parasites ingested by a mosquito reduces its survival. If this is the case then a lower infection intensity, without affecting prevalence, would increase the fitness of infected mosquitoes while at the same time increasing the parasites chance of being transmitted. If the fitness costs are confirmed, this suggests local adaptation of the vector to the parasite and parasite to the vector, which has strong implications for malaria transmission dynamics.

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THE EVOLUTIONARY CONSEQUENCES OF HOST SPECIES CHOICE FOR AFRICAN MALARIA VECTORS: COULD UNTREATED BED NETS SELECT FOR A HOST SHIFT?

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The host preference of malaria vectors is one of the key determinants of global transmission patterns. Here we conducted an experimental investigation of the major African malaria vectors Anopheles gambiae s.s and An. arabiensis to test whether their preference for humans over other commonly available animal hosts can be explained by the fitness benefits they derive from them, and whether the use of common interventions such as bednets can reduce the advantage of anthrophily to the point where selection for a host species shift could be generated. Experiments were conducted in which one host of either cow, human (exposed or protected by untreated net), dog, goat or chicken was placed inside an experimental hut set within a unique Semi-Field System (SFS) at Ifakara Health Institute in Tanzania. Groups of 200 insectary-reared An. gambiae s.s or An. arabiensis were released into the chamber at dusk and left overnight with the host. The next morning mosquitoes were recaptured and their blood feeding success and subsequent fecundity and survival measured (6 replicates of each host and vector species combination).

Whereas Anopheles arabiensis had a significantly greater feeding success on its naturally preferred bovid hosts, the more anthrophilic An. gambiae s.s. took considerably bigger blood meals and had greater survival after feeding on human than animal blood. The use of a bednet failed to completely prevent biting by either vector, but did reduce the expected fitness benefits from human hosts. By modeling the combined effect of all host species impacts on vector fitness, a mosquito life-history model predicted the lifetime egg production of An. arabiensis to be considerably higher on their naturally preferred bovids than all other host types. However, the lifetime reproductive success of the naturally anthrophilic An. gambiae s.s. was not predicted to be higher on humans than any other host species. Further study is required to identify the nature of selection favouring anthrophily in this vector, and possibilities for reducing it through vector control and/or environmental management.

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QUANTIFYING AND ANALYZING DANCE OF *ANOPHELES GAMBIAE* IN MATING SWARMS

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We describe a technological breakthrough achieved through interdisciplinary collaboration that allows us to track individual swarming *Anopheles gambiae* males and females from stereoscopic video footage obtained from the field in Mali, Africa in August 2009. Mating behaviors of malaria vectors in nature are rarely described or studied, and even more rarely quantified. The actual movement of individual members of a mating swarm of mosquitoes over time has never before been measured. The principal reason this stage of the life history remains unexplored is that direct observation and quantification of mating in a swarm is very difficult due to the small size of mosquitoes, their relatively fast rate of movement and their habit of aggregating under conditions of low light. Despite our lack of knowledge in this area, determinants of male mating success in

medically important species are of major interest from a fundamental and applied perspective. We estimate three-dimensional position and velocity of individual mosquitoes through space and time by employing advanced image processing and probabilistic estimation techniques in a semi-automated computer tracking system. We verify the accuracy of our tracking system along several parameters by comparing it with manually created ground-truth data. We have used the system to quantify and analyze three instances of couple formation in *An. gambiae* swarms. These measurements reveal that female *An. gambiae* spend much more time in a swarm composed almost entirely of males than previously thought: more than six seconds in one instance. We will describe ongoing studies in which we are quantifying the differences in the flight patterns of males and females within the mating swarm and studying male flight patterns for determinants of male mating success. These data and the increasingly automated capacity in which we generate them promise to greatly extend our fundamental understanding of *An. gambiae* mating biology and have implications for any release-based approach to its control as a malaria vector.

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IMPROVING AVAILABILITY OF KEY ANTIMALARIAL COMMODITIES: PILOTING AN ESSENTIAL DRUG LOGISTICS SYSTEM IN ZAMBIA

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The successful implementation of malaria programs depends on a continuous supply of malaria-related commodities: artemisinin-based combination therapy (ACTs), rapid diagnostic tests (RDTs), sulphadoxine-pyrimethamine (SP), and quinine. From January 2009 to January 2010, the United States Agency for International Development | DELIVER PROJECT supported the Ministry of Health of Zambia when they conducted an essential drugs/malaria logistics systems pilot to determine what key logistics factors impact commodity availability and to identify and implement the most effective elements to ultimately improve efficiency in the supply chain. To ensure statistical power, the districts were selected using a random stratified technique; eight districts per pilot system were selected. Twenty-four districts were involved, including a control group of eight districts. Some of the factors used in the sampling included prevalence of malaria, accessibility, urban/rural factor, and number of facilities. With support from the United States Agency for International Development | DELIVER PROJECT, a steering committee of key partners designed and piloted two logistics systems. In one system, the district serves as a pass-through to distribute commodities that are pre-packaged by the central medical stores. In the other, the district stores, packages, and supplies the facilities directly. The two pilot systems were rolled out in 16 districts and 347 service delivery sites. Specific variables tested were the availability of antimalarial drugs, transport from district to facility, storage capacity, and commodity management at the district- and health-facility level. To determine the impact on stock availability in each of the pilots, teams conducted a final evaluation visit to 259 sites, which were randomly chosen. The results of the assessment, completed in March 2010, showed that introducing a logistics system drastically reduced the stockout levels of antimalarial products, even when some products had stockouts at the central level. For example, pediatric ACTs were available in 88% of the pilot facilities, compared with 51% in the control; they were available 345 days a year, compared with 247 days in the control.

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FIVE-YEAR OVERVIEW OF STRENGTHENING REGIONAL CAPACITY IN MEDICINES QUALITY IN THE MEKONG SUBREGION: A FOCUS ON COUNTERFEIT AND SUBSTANDARD MEDICINES

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In the Mekong subregion, an estimated 10-35% of medicines are improperly made or illegally imported and sold. To help country authorities in this region address medicine quality problems, in collaboration with its partners, the Promoting the Quality of Medicines (PQM) program - funded by the United States Agency for International Development (United States Agency for International Development) and implemented by the United States Pharmacopeia (USP) - has introduced a regional mechanism for Medicine Quality Monitoring (MQM). The MQM program focuses on early detection of poor-quality antimalarial, anti-TB, antibiotic, antiretroviral, and Avian Influenza medicines and strengthening the capacity of regulatory authorities for enforcement actions based on evidence obtained from the field. The ultimate goal is to reduce the prevalence of poor-quality medicines_counterfeit and substandard_available in the public, private, and informal sectors in Cambodia, Lao PDR, Thailand, and Vietnam. A well-designed framework and protocol for sampling, testing, data management and reporting were used. Data collected in 2004 revealed the wide availability of poor quality medicines: in some Mekong countries, up to 44% of artesunate (a commonly used, highly efficacious antimalarial) samples collected and tested contained no active ingredient. In 2008, this figure dropped to 11.2%. From 2005-2009 in the Mekong Subregion, the MQM program sampled 3,021 antibiotic, 6,176 antimalarial, 625 anti-tuberculosis, and 234 antiretroviral medicines. Antibiotics and antimalarials had the highest failure rate of 2.3% and 2% respectively. To present, the total number of samples collected and tested has reached almost 4,000. While initial failure rates in the region averaged around 6%, there was a steady trend toward a decreasing prevalence at the 42 monitoring sentinel sites in the region and in 2009, the overall failure was 1.3%. Medicines quality data were used to raise public awareness and shared with national law enforcement agencies for appropriate actions. They were also shared with international agencies like World Health Organization and INTERPOL, for collective investigations and actions at regional and international levels. Despite these progresses and achievements, many challenges remain to be addressed and the MQM needs to be scaled up to cover the whole region.

1302

CHANGING GUIDELINES FOR MALARIA CASE MANAGEMENT: CAN HEALTH FACILITIES KEEP UP?

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The World Health Organization (WHO) has recently released new guidelines for treatment of malaria, recommending that suspected cases be confirmed by a parasitological test when possible. However, the current capacity for diagnosis and management of malaria and other febrile illnesses within the existing health system is unclear. To inform the design of future interventions, we conducted a situational analysis of government-run health centers in rural Eastern Uganda. Health workers stationed at centers in five sub-counties in Tororo district were approached for study participation. Structured questionnaires

addressing staffing, training and supervision, and knowledge of malaria case management were administered to consenting health workers. We interviewed 81 (88%) of 92 health workers stationed at the 17 local health centers, representing the spectrum of staff cadres. Staffing shortages were a problem at nearly all health centers. Unpaid volunteers with limited medical training constituted 26% of staff interviewed, and 48% at the lowest-level health centers. Most health workers (56 [69%]) were trained in management of malaria with artemether-lumefantrine, but only 29 (26%) had received training in rapid diagnostic tests (RDTs) for malaria. Overall, knowledge about malaria case management was poor; mean knowledge scores ranged from 18% (vaccinators) to 45% (nursing officers). The in-charges of health centers scored surprisingly low, as did volunteers (mean scores 34% and 20%, respectively). When asked how to confirm the diagnosis of malaria, only eight (10%) health workers mentioned microscopy and two (2%) RDTs. Additional gaps in knowledge included recognition of danger signs for severe malaria, differential diagnosis of non-malarial febrile illnesses, and key elements of managing and treating malaria. The shift towards universal diagnostic testing for malaria is a major step to ensuring appropriate management of malaria and other febrile illnesses. We found that health centers in Eastern Uganda currently have limited capacity to adhere to WHO's new guidelines. Poor staffing of health centres and gaps in knowledge urgently need to be addressed.

1303

VOLUNTEER COMMUNITY HEALTH WORKERS: TEMPORARY FIX OR LONG-TERM SOLUTION?

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Community management of febrile children is evolving away from providing treatment of malaria only, toward a more integrated approach, focusing on malaria, pneumonia and diarrhea. However, more complex programmes place higher expectations on community health workers (CHWs). In Uganda, a policy of integrated community case management (iCCM) is being adopted. To investigate current challenges faced by CHWs, we conducted a situation analysis in rural Eastern Uganda. We interviewed 100 CHWs selected using convenience sampling from five sub-counties in Tororo district. A structured questionnaire addressing patient load was administered, and in-depth interviews were conducted. Focus group discussions were also conducted with health workers and community members. The CHWs reported receiving close to 20 patients per week (mean 19.5, SD 12.2, range 2-63), including 15 malaria patients (mean 15, SD 10.8). Only ten CHWs reported receiving specific incentives, such as payment, to motivate them to perform their duties. Interviews revealed that over 80% of CHWs were motivated by non-monetary incentives including altruistic reasons, good relationships with health workers, and self benefit, with the strongest theme focusing on respect or 'becoming someone important' through their work. However, over half of CHWs felt demotivated due to limited support from communities and the health system, unrealistic expectations of caregivers, lack of drugs and supplies, and lack of compensation. Although communities appeared to understand the role of CHWs, they often inappropriately attributed the logistical constraints and lack of CHW motivation to personal rather than system shortfalls. Our results suggest that many volunteer CHWs in Uganda are motivated by altruistic and/or self-serving motives. However, the non-monetary benefits of becoming a CHW, including social status, appear to wane after one year. Relying on minimally trained volunteers to deliver community programmes may temporarily address gaps in the health system but is unlikely to be a sustainable solution. Focus on community programmes should not divert attention away from longer-term interventions to improve health care infrastructure and delivery of good quality health care by trained practitioners.

1304

DEMYSTIFYING DRUG DELIVERY: A ONE-PAGE SOLUTION

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Drug stock-outs due to poorly functioning delivery systems are a major barrier to providing good quality health care in many resource-poor settings. In Uganda, stock-outs of artemether-lumefantrine (AL) threaten strategies to reduce malaria-related morbidity and mortality. Complex, large-scale initiatives have been suggested to improve health systems; however, few inexpensive, simple strategies exist to improve drug delivery at the periphery. To investigate challenges and possible solutions for drug delivery in Uganda, structured questionnaires were administered to representatives of 17 government-run health centers in Tororo district. We also conducted a literature review, in-depth interviews, and focus group discussions (FGDs) with health workers and key informants. Drug stock-outs are a major problem at most health centers, particularly at the lowest level. Two (12%) centers never stock AL, and only 13 (76%) had AL in stock on the day of the interview. Stock-outs of other antimalarial drugs, such as oral and injectable quinine and artesunate + amodiaquine, and other medications including panadol, amoxicillin, mebendazole, oral rehydration solution, and iron were also reported in most health centers. The literature review revealed several challenges to drug delivery including managerial inefficiencies, limitations in human resources, and logistical constraints. The interviews and FGDs revealed that health workers lack knowledge about the drug delivery system and are confused about the roles and responsibilities of staff involved in drug procurement. Challenges including inadequate transport, lack of funding, and delays in processing orders were cited as reasons for stock-outs. Most health workers agree that failure to provide drugs results in unmet expectations and poor quality health care. The challenges identified represent logistical nodes which should be operational to ensure effective and efficient drug delivery. We have developed a simple one-page tool based on formative evaluation and logistics measurement methodologies to help identify, communicate and resolve drug distribution issues. We plan to implement and evaluate the tool in a cluster-randomized control trial in Uganda.

1305

ANTIBIOTIC USE IN INFANTS IN A SETTING WHERE ANTIBIOTICS ARE AVAILABLE WITHOUT PRESCRIPTION: WHOSE RESPONSIBILITY?

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Antibiotics are the most commonly prescribed drugs in children. However their misuse has been associated with the development of resistant pathogens. Generally, it has been assumed that the availability of antibiotics without medical advice has been one of the most important causes of misuse. The aim of this study was to describe the use of antibiotics in Peruvian infants in a setting where antibiotics are available without prescription. Within a cohort study of 1023 children of peri-urban Lima, antibiotic use data were recorded in the clinical records at a study clinic. Children less than 2 months of age were enrolled and followed until 12 months of age. History of previous illnesses and drug use prior to enrollment and between the scheduled visits was recorded in the medical

record as well as the treatment offered by the study physician. Clinical records were reviewed and descriptive analysis was performed. During a one year period, 770 of 1023 (75.3%) children took 2085 courses of antibiotics. There were two courses/child/year, range (0-12). The mean age of usage was 6.5 months (range 9 days to 12.9 months). Higher rates of antibiotic use were found in children from 3 to 6 months of age (37.2%). Antibiotics were given to children in 8.5% of common colds, 59.1% of all pharyngitis, 68.9% of bronchitis, 64.4% of diarrheas, 23% of dermatitis, and 12% of bronchial obstruction. Physician prescription was the most common reason for antibiotic use (90.8%), self-medication was found in 6.9% and was preceded by a physician antibiotic prescription in 63.9%. The most frequently used antibiotics were penicillins (32.9%) and macrolides (23.4%). Upper respiratory tract infections were treated mainly with penicillins (56.4%), diarrhea with macrolides (49.6%) and bronchial obstruction with penicillins (44.6%). Based on the diagnoses 83.1% of the antibiotic prescribed drugs were inappropriate. In conclusion, infants are often exposed to antibiotics early in life in this peri-urban area. Antibiotic use is typically inappropriate (83.1% of courses) based on the most common etiologies for this age-group. Since physicians prescription was the most common reason for antibiotic use, interventions to improve use of antibiotics should focus on them.

1306

FEASIBILITY OF WEB-BASED TELECONFERENCING FOR REMOTE ULTRASOUND TRAINING AND QUALITY ASSURANCE IN REMOTE SETTINGS

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Ultrasound (US) is an important adjunct to diagnosis and management in resource-limited settings. However, US is highly operator dependent and in remote locations, ongoing training is difficult. The objective of this study was to determine the feasibility of remote US training and quality assurance (QA) in a remote, resource-limited setting. Ultrasound training was provided to physicians working in the developing world using a SonoSite 180 Plus US machine with a C60 broadband curved array transducer. The investigators reviewed selected exams obtained on patients remotely as well as conducting on-line real time QA and educational sessions. Cases were reviewed from remote locations in South America and South-East Asia using two methods of web-based video conferencing: Skype and ooVoo. Skype worked well for one-on-one training using ultrasound images only, but did not allow for ultrasound video conferencing. However, the ultrasound equipment only allowed for still image archive. Skype also did not allow for PowerPoint based presentations. OoVoo allowed for video conferencing at more than one site. In this case, 4 sites were imaged simultaneously in 2 countries with streaming-video. OoVoo allowed for PowerPoint based presentations to be viewed by all participants. OoVoo required a faster Internet connection and one site had a long delay in the video images (Cambodia). Video conferencing allowed for training in patient positioning, sonographic windows, and also suggestions in improvement in scanning techniques. Review of one scan resulted in a change in diagnosis, which was significant, diagnosis of gallbladder cancer. The video conferencing using Skype from South America went well without loss of signal. With multiple video conferencing using ooVoo, there was a transmission delay, which was most pronounced in the South-East Asia site and resulted in loss of connection 3 times over the 90-minute session. In conclusion, web-based QA and ongoing training in US with remote, resource-limited sites is possible using commercially available programs.

1307

MOSQUITO CELLS SURVIVE FROM DENGUE 2 VIRUS INFECTION THROUGH AN ANTI-OXIDANT DEFENSE

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Both mosquito and mammalian cells are essential for the propagation of dengue (Den) viruses during alternate transmission of the viruses. The viruses generally induce apoptosis in mammalian cells but cause only minor damages in mosquito cells, leading to persistent infection in most cases. In order to find genes involved in determining the cell fate, datasets derived from expressed sequence tags (ESTs) of C6/36 cells with and without infection were established. A total of 3876 unigenes which contained 875 contigs and 3001 singletons were obtained. Of which, 2267 and 2189 unigenes were respectively expressed in mock- and infected complementary DNA (cDNA) libraries. Among the generated unigenes, 600 (in addition to 7 viral proteins) were only found in infected cells, while 642 were only found in mock-infected cells. Chaperone protein including GRP78/BiP and endoplasmin were found to be significantly upregulated in C6/36 cells infected by Den-2 virus for 24 h. This suggests that Den-2 virus infection in mosquito cells, as in mammalian cells, activates the unfolded protein response (UPR) to cope with the endoplasmic reticulum (ER) stress at the early stage of infection. Changes of mitochondria membrane potential (MMP) and generation of superoxide provided further evidence that Den-2 virus induce the oxidative stress in spite most infected cells remain intact. Due to significant elevation of the superoxide dismutase (SOD) activity, mosquito cells are able to rescue themselves from viral infection through antioxidant defenses. The findings of this study have shed lights on interactions between the virus and host cells, particularly mosquito cells.

1308

DENGUE-2 ALTERS SALIVARY GLAND PROTEIN EXPRESSION IN INFECTED *AEDES AEGYPTI* MOSQUITOES

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The principle arthropod vector of dengue virus is the *Aedes aegypti* mosquito, and in that vector the virus must disseminate to the salivary glands prior to being transmitted to the vertebrate host via salivation during probing and/or feeding. As has been shown previously, the saliva of mosquitoes contains a diverse cocktail of pharmacologically active compounds that are deposited simultaneously with the virus to the bite site of the vertebrate host. It is here that the mosquito's saliva modifies the local environment, perhaps in a way that facilitates the establishment of an infection. In order to determine whether dengue virus infection alters the protein composition of the saliva in the mosquito, we have analyzed the proteins expressed in *Aedes aegypti* salivary glands in infected mosquitoes and uninfected control mosquitoes via 2-D gel electrophoresis. Briefly, *Aedes aegypti* (Rockefeller) were orally exposed to bovine blood in Alsever's with and without dengue-2 (strain 1232). Mosquitoes were held for 9 days at 28° C before salivary glands were dissected; total proteins were extracted, and separated by 2-D electrophoresis. Mass spectroscopy analysis (LC-MS/MS) revealed that several proteins were differentially expressed between the two cohorts. In particular are several down-regulated proteins involved in ATP synthesis. We also have observed a decrease in a 30kD allergen protein and an increase in D7 in infected mosquito salivary glands, leading to an altered salivary composition that may create a more receptive environment for a successful viral infection This research indicates the need to not only review the components of mosquito saliva that are being inoculated with the virus, but also highlights the potential direct effects the virus may have on the composition of the saliva due to salivary gland structural and functional changes in the infected invertebrate.

1309

VIRAL AND IMMUNOLOGICAL DETERMINANTS OF DENGUE VIRUS FITNESS AND VIRULENCE

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Four serotypes of dengue virus (DENV1-4) circulate in humans, causing more illness than any other arthropod-borne virus. Despite decades of epidemiological research, we lack a sufficient understanding of the relative importance of host genetics, pre-existing immunity and viral evolution in dengue virus pathogenesis. We report here a significant increase in the incidence of severe dengue disease caused by DENV-2 in two independent studies of pediatric dengue in Managua, Nicaragua. Through full-length genome sequencing of viruses isolated from patients across several years (2005-2008), the increase in severity was found to be correlated with a clade replacement event occurring in DENV-2 circulating during the same time frame. Clade assignment by genotyping methods of additional viruses whose full-length sequence was not available increased the initial sample size substantially. Association analyses including clade and year suggests that a shift in viral genetics does not explain the increased severity observed in the later years of the studies. However, viral isolates derived from the replacing clade ("Clade 2") replicate more productively *in vitro* in human and mosquito cells, indicating that clade replacement involved the evolution of more fit DENV-2 viruses. Thus, our findings support a model in which increased viral fitness is not necessarily linked to increased pathogenesis. Consistent with this model, more in-depth analyses of clinical indicators of severity, such as low platelet count and hemoconcentration, suggest that less fit viruses ("Clade 1") are associated with more severe disease outcomes when stratified by year. Finally, we are exploring the alternative hypothesis that waning cross-reactive immunity in the population resulting from infection with a heterologous serotype (DENV-1, which circulated in Managua in 2003-5) is sufficient to explain the increased severity associated with DENV-2 infections in later years (2006-8) and are testing this by analyzing neutralization profiles of serum samples collected from our pediatric cohort study in 2004-2008. Our findings provide the first in-depth analysis of the contribution of relatively small genetic changes in viral sequence to viral fitness and pathogenicity and suggest that pre-existing immunity is the major determinant of dengue virus pathogenesis.

1310

GENETIC AND PHENOTYPIC CHARACTERIZATION OF SYLVATIC DENGUE VIRUS TYPE 4 STRAINS

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The four serotypes of endemic dengue viruses (DENV) circulate between humans and peridomestic *Aedes* mosquitoes. At present endemic DENV infect 100 million people per year, and a third of the global population is at risk. In contrast, sylvatic DENV strains are maintained in a transmission cycle between nonhuman primates and sylvatic *Aedes* species, and are evolutionarily and ecologically distinct from endemic DENV strains. Phylogenetic analyses place sylvatic strains basal to each of the endemic serotypes, supporting the hypothesis that each of the endemic DENV serotypes emerged independently from sylvatic ancestors. We utilized complete genome analyses of both sylvatic and endemic DENV serotype 4 (DENV-4) to expand our understanding of their genetic relationships. A high degree of conservation was observed in both the 5'- and 3'-untranslated genome regions, whereas considerable differences at the nucleotide and amino acid levels were observed within the open reading frame. Additionally, replication of the two genotypes was compared in mammalian (Vero and Huh-7) and mosquito (C6/36) cultured cells. Understanding the genetic relationships and phenotypic differences between endemic and sylvatic DENV genotypes may provide valuable insight into DENV emergence and guide monitoring of future outbreaks.

1311

FAST GROWTH DENGUE-LIKE CHIMERIC VIRUSES

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Four chimeric West Nile/dengue viruses were engineered by expressing the premembrane-envelope (prM-E) gene region of dengue serotypes 1-4 (DEN 1-4) in the genetic backbone of West Nile virus. These viruses were stabilized by incorporating mutations which enhanced the fitness of the chimeras in Vero cells. They exhibited DEN serotype-specific antigenic properties and produced clear immune foci and plaques in Vero cell monolayers within 1 and 3 days, respectively. Compared to wild-type DEN viruses, these DEN-like chimeras replicated rapidly and reached peak titers at 3-5 days earlier in Vero cells and 2 days earlier in C6/36 cells. They achieved similar or higher titers than wild-type DEN viruses in the cell cultures. Despite their higher replication efficiency, these DEN-like chimeric viruses were attenuated in mice and had poor dissemination rates in mosquitoes. Due to their fast growth and attenuated properties, these viruses may be useful in various aspects of vaccine development, in diagnostic assays, and in DEN virus research.

1312

ACCELERATED PLATELET APOPTOSIS IS ASSOCIATED WITH PLATELET PHAGOCYTOSIS AND THROMBOCYTOPENIA IN SECONDARY DENGUE VIRUS INFECTION

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An increased platelet phagocytosis was demonstrated during the acute phase of patients with secondary dengue virus (DV) infection, as reported previously. To determine the role of apoptosis in the phagocytosis of platelets, the relationship between platelet phagocytosis by differentiated THP-1 macrophages and platelet apoptosis was examined by flowcytometry using freshly isolated platelets from 32 patients clinically diagnosed with DV infection at San Lazaro Hospital, Manila, Philippines in year 2009.

The levels of platelet apoptosis from patients were significantly increased during the acute and early convalescent phase of infection compared with those of the convalescent phase and healthy controls. In addition, a significant inverse correlation was found between the peripheral platelet counts, the levels of platelet apoptosis (by Annexin V binding: $r = -0.491$, $p = 0.001$; by caspase-3 activation: $r = -0.507$, $p = 0.001$) and and the levels of platelet phagocytosis ($r = -0.455$, $p = 0.002$) among these patients. Furthermore, a significant direct correlation between the levels of platelet phagocytosis and platelet apoptosis (against Annexin V binding: $r = 0.395$, $p = 0.007$; against caspase-3 activation: $r = 0.453$, $p = 0.002$) was also found in these patients. Meanwhile, no effects were observed upon utilizing anti-Fc receptor IgG or anti-CR3 IgG as inhibitors in the phagocytosis of platelets by macrophages.

Collectively, our data suggest that accelerated phagocytosis of apoptotic platelets is involved in the mechanisms of thrombocytopenia in secondary DV infection. Further studies on the mechanisms of platelet apoptosis and platelet phagocytosis during the acute phase of secondary DV infection are warranted.

1313

PERMISSIVENESS OF BONE MARROW CELLS FOR DENGUE VIRUS INFECTION IS AGE-DEPENDENT

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Dengue is one of the most important mosquito-borne viral diseases affecting humans, with over half of the world's population living in areas at risk. Bone marrow suppression has been observed in dengue patients during the acute stage of infection associated with reduction of megakaryocytes. Studies of bone marrow biopsies from patients during acute infection indicate dengue virus infection induces bone marrow progenitor cells hypocellularity. Results from early attempts to investigate the possible underlying mechanisms leading to bone marrow suppression *in vitro* have been inconclusive. A systematic investigation on this subject was performed with bone marrow from 10 rhesus monkeys of various ages. Freshly collected bone marrow aspirates were infected with low dose of dengue virus, strain 16881 grown in Vero cells, at MOI=0.1. Cell smears were performed and supernatant fluids were collected daily for 10 consecutive days. Quantitative real-time RT-PCR was used to measure the viral titers in the supernatant fluids and immunohistochemistry staining with antibodies for cellular surface markers and dengue viral antigen was performed on smears. Results revealed that bone marrow from i) young monkeys (under 5 years old) were highly permissive to infection and able to support dengue virus replication with viral titers peaking at 2-3 days after infection; ii) older monkeys (over 5 years old) generated two patterns; viral titers declined from day 1 to day 10 and occasionally, viral titer peaked at 1 day after infection. Surface markers staining of sequential daily samples indicated that progenitor cells expressing CD235a and CD41 markers were positive for dengue viral antigen early (1-3 days) post infection, while cells with markers typical for dendritic cells or macrophages were positive for dengue viral antigen at later days (5-8) of infection. The significance of the findings will be discussed.

1314

COMMUNITY-BASED DELIVERY OF HEALTH CARE: WHAT IS THE CAPACITY FOR EXPANDING INTERVENTIONS?

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Prompt treatment with effective antimalarial drugs is one of the key strategies for reducing the burden of malaria, and community-based programs are advocated to improve access to treatment. In 2002, Uganda adopted a policy of home management of malaria (HMM), which is now scaling-up to integrated community case management (iCCM). Community health workers (CHWs) will provide presumptive treatment for malaria, pneumonia, and diarrhea to febrile children. To investigate challenges faced by CHWs in the existing HMM programme, we conducted a situation analysis in rural Eastern Uganda. We interviewed 100 CHWs selected using convenience sampling from five sub-counties in Tororo district. A structured questionnaire addressing training and supervision and a knowledge questionnaire were administered, and in-depth interviews were conducted. We identified major gaps in CHW training, knowledge, and supervision. Overall, CHWs scored poorly on the knowledge questionnaire (mean score 22%). Only 74% CHWs correctly identified fever as the most common symptom of malaria in children, and recognition of danger signs of severe malaria was poor. Although 61% of CHWs had received training on management of malaria with artemether-lumefantrine (AL), few CHWs correctly described how AL should be administered. Only 23% said that they would refer a child who was not improving after two days. Recognition of non-malarial causes of fever in children was also poor. Only four CHWs reported receiving support supervision in the last six months. Interviews revealed that CMDs are involved in implementing multiple programmes led by different stakeholders, which are not integrated. Community-based programs provide opportunities to improve access to treatment, but their success depends on the capacity of CHWs to implement strategies. In Uganda, CHWs knowledge of appropriate management of malaria is limited, despite training, and they may be overstretched by stakeholders attempting to deliver community-based interventions. CHWs may lack capacity to deliver complex interventions successfully and sustainably. To ensure interventions are implemented appropriately, attention should be paid to training and support supervision of CHWs, and evaluation of the health impact of programmes.

1315

SUBOPTIMAL MANAGEMENT OF SEVERE MALARIA CASES IN UGANDAN HEALTH FACILITIES: A CROSS SECTIONAL SURVEY

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Malaria morbidity and mortality in Africa remains unacceptably high, partly due to sub-optimal case management. We evaluated the management practices of severe malaria in Ugandan health facilities by conducting a cross sectional survey using multi-stage sampling methods. Health facilities were selected in 11 districts in the eastern and mid-western parts of the country. The study instruments were adapted from the WHO hospital care assessment tools. Between June and August 2009, 105 health facilities were surveyed and 181 health workers and 868 patient/caregivers were interviewed. None of the inpatient facilities had all seven components of the basic care package for the management of severe malaria consistently available during the 3 months prior to the survey. Referral practices were appropriate in less than 10% (18/196) of the patients, while prompt care was reported by 29% (247/868) of the patients. Severe malaria was correctly diagnosed in 27 % (233/868) of the patients. Though the quinine dose and regimen was correct in the majority of patients (611/868, 70%), it was administered in the correct volumes of 5 % dextrose in only 18% (147/815) of the cases. Most patients (80%) had several doses of quinine administered in one single 500ml bottle of 5% dextrose. Medications were purchased by 385 (44%) patients and medical supplies by 478 patients (71%). These results highlight the challenges of correctly managing severe malaria in Uganda and other resource limited settings. Several priority areas need improvement, including triage and emergency care, referral practices, quality of diagnosis and treatment, health worker training, zero tolerance for stock outs for recommended medicines and ancillary treatments, functional referral systems, adequate infrastructure, better organization of hospital services, and regular supervision and clinical audits.

1316

AN ASSESSMENT OF ADHERENCE TO ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN PHALOMBE, MALAWI, 2009

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Malaria causes substantial morbidity and mortality in Malawi. Prompt and effective treatment is a cornerstone of malaria control. In 2007, due to increasing parasite resistance to the first-line treatment for uncomplicated malaria, Malawi replaced single-dose sulphadoxine-pyrimethamine treatment with a six-dose, three-day treatment regimen of artemether-lumefantrine (AL). Given concerns about the complex AL regimen, we assessed patient adherence to AL for the treatment of uncomplicated malaria in Phalombe District, Malawi. Adults and children with uncomplicated malaria were recruited at three health centers. To assess adherence, we conducted pill counts and in-home interviews on medication consumption 72-hours after patients received AL. Complete adherence was defined as correctly taking all six doses of AL as assessed by pill count and patient recall of number of doses, number of pills per dose, and timing for each dose. We recruited 427 patients, completed in-home interviews on 414 (97%), and analyzed 368 (86%) patients with complete data. Among 368 patients, 238 (65%) were completely adherent. Classifications of non-adherence included skipping doses, taking incorrect number of pills per dose, or taking doses at incorrect times. Factors significantly associated with adherence were a preference for AL over other medications (odds ratio (OR) 2.7, p<0.001), use of AL package for instructions, (OR 2.5, p=0.02), and direct observation of the first dose of AL (OR 2.4, p<0.01). In contrast, being <5 years old was associated with non-adherence (OR 0.5, p=0.05). In conclusion, two-thirds of patients assessed were completely adherent to a six-dose AL regimen for the treatment of uncomplicated malaria. Efforts to improve adherence should focus on children <5 years old, the age-group most vulnerable to malaria. Interventions including direct observation of the first dose, utilization of the AL package for instructions, and enhancing patient preference for AL have the potential to increase adherence and therefore improve cure rates, and possibly mitigate antimalarial drug resistance.

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MAPPING THE PRIVATE SECTOR SUPPLY CHAIN TO UNDERSTAND THE RETAIL PRICES AND AVAILABILITY OF ANTIMALARIALS IN SIX LOW-INCOME COUNTRIES IN AFRICA AND SOUTH EAST ASIA

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In many low-income settings the private sector plays a crucial role in the delivery of antimalarials, often complementing the formal public health system. In the context of the introduction of a global subsidy to improve access to artemisinin-based combination therapies (ACTs) it is important to understand private sector supply chains and how they vary across countries, as these have an important impact on price and availability, and therefore equitable access. As part of the ACTwatch study, we undertook surveys of nationally representative samples of private sector antimalarial wholesalers and retailers, collecting volume, mark-up and provider characteristic data in 6 countries (Benin, Cambodia, DR Congo, Nigeria, Uganda, and Zambia). In total, structured interviews were completed with 688 wholesalers and 7048 retailers across the 6 countries between February 2009 and April 2010. We will present maps of the private sector antimalarial supply chains in each country, descriptions of their composition and characteristics, and estimates of availability and price mark-ups for antimalarials. For example, 81.8% of Zambian wholesalers had antimalarials available, while only 64.7% had an ACT in stock, 40.0% had an artemisinin monotherapy (AMT) and 60.0% had a non-artemisinin therapy (e.g. chloroquine) in stock at the time of interview. In terms of percentage price mark-up on ACTs, median wholesaler mark-ups (26.7%) were observed to be generally lower than median retail level mark-ups, which ranged from 42.9% in private pharmacies to 150% in grocery stores. Median percentage price mark-ups in Zambia for AMTs were similar to those for ACTs, 26.1% among wholesalers, and a range of 42.9% to 168% among private sector retailers. Results across countries will be contrasted and implications for interventions to improve ACT access through the private sector, such as the Affordable Medicines Facility for Malaria, will be explored.

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CHANGES IN MALARIA IN SOUTHERN SENEGAL WITH THE INTRODUCTION OF ARTESUNATE PLUS AMODIAQUINE AND PARASITOLOGICAL DIAGNOSIS

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In Senegal, antimalarial treatment policy changed from chloroquine or quinine on clinical grounds to artesunate/amodiaquine (ASAQ) on parasitological confirmation in 2006. RDTs are provided from 2007. In the District of Oussouye, the new policy was staggered in since 2000 in Mlomp and later elsewhere. Malaria is meso-endemic with transmission peaking July-December (EIR 25 in 2000). Data for 1996-2008 were extracted from the clinic registries of the referral health centre and the four peripheral dispensaries of the District of Oussouye. Data over time were analysed by logistic regression. Pluviometry and bednet distribution were also accounted. Over the entire 13-year period a total of 363,966 consultations occurred (mean 1.2 person/year) with 139,144 antimalarial treatments (on either clinical or parasitological grounds; 38% of all attendances) and a projected 32,384 true cases of malaria (~77% of antimalarial treatments were redundant.) In Mlomp (early implementation) compared to 1996, the number of consultations, antimalarial treatments and estimated malaria incidence increased initially until 1998/2000 and then decreased steadily to reach in 2008 OR (95%CI) of 0.39 (0.38-0.41), 0.18 (0.17-0.20) and 0.09 (0.08-0.11) respectively vs. 1996. The age of malaria patients changed over time; while the proportion of patients 0-5 and 6-10 years old decreased steadily and 11-15 remained overall constant, all categories >15 increased. In 2008, all categories >10 were all at higher risk than the 0-5 years (in particular, the RR(95%CI) for >30 years was 14 (1.5-131.7) Details on the comparative data from the other sites will be presented. In conclusion, there is a temporal correlation between the implementation of ASAQ and parasitological confirmation and decreasing consultations to the health facilities, fevers diagnosed and treated as malaria and estimated malaria incidence in this meso-endemic area. The age profile changes are compatible with decreased transmission intensity.

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AN EVALUATION OF THE EFFECT OF TRIMETHOPRIM-SULFAMETHOXAZOLE PROPHYLAXIS ON GAMETOCYTEMIA AND ASYMPTOMATIC PARASITEMIA IN HIV-EXPOSED CHILDREN IN RURAL UGANDA

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Treatment with sulfadoxine-pyrimethamine (SP), an antifolate antimalarial, has been associated with increased gametocytemia, especially in the presence of antifolate resistance. However, data are limited regarding the effect of trimethoprim-sulfamethoxazole (TS) prophylaxis on gametocytemia. We previously reported that TS prophylaxis has a 40% protective efficacy against malaria among HIV-exposed (HIV-uninfected infants born to HIV-infected mothers) children, but data are lacking concerning the effect of TS on asymptomatic parasitemia (AP). Here, we examine the effects of TS prophylaxis on gametocytemia and AP. In an area of high malaria endemicity and antifolate resistance in rural Uganda, we randomized 185 HIV-exposed infants (median age= 9.6 months), following breastfeeding and a negative HIV PCR test, to discontinue or continue TS prophylaxis through age 2 years. Routine smears were obtained every 30 days, and time-at-risk was divided into calendar months. Gametocytemia and AP were diagnosed by microscopy (and absence of fever for AP). All smears performed within 7 days of a malaria episode and during malaria follow-up were censored in assessing for prevalence of AP. Among 98 infants randomized to continue TS, there were 28 episodes of gametocytemia over 1,068 months (2.6%), and among 87 infants randomized to stop TS, there were 8 episodes of gametocytemia over 845 months (1.0%) (RR=2.83, p=0.07). Among children taking TS, there were 77 AP episodes over 812 months (9.5%), and among children who discontinued TS, there were 78 AP episodes over 606 months (12.9%) (RR=0.74, p=0.18). Compared to AP in participants not taking TS, AP in children taking TS prophylaxis was less likely to progress to malaria within 7 days (RR=0.41, p=0.001). Though the overall prevalence of gametocytes was low, TS prophylaxis was associated with a trend toward increasing gametocytemia. Compared to children not taking TS, AP episodes among children taking TS prophylaxis were less likely to progress to clinical malaria, indicating that TS may prevent malaria at the erythrocytic stage.

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INTERMITTENT PREVENTIVE THERAPY IN PREGNANCY WITH SULPHADOXINE-PYRIMETHAMINE (SP); 42 DAY *IN-VIVO* FOLLOW-UP STUDY AMONG ASYMPTOMATIC PARASITEMIC PREGNANT WOMEN IN AN AREA WITH HIGH SP RESISTANCE IN SOUTHERN 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 during 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 has remained effective for IPTp in many areas of Africa. We conducted a 42-day in-vivo assessment of the parasitological response to IPTp-SP in women scheduled to receive their first dose of IPTp, to study the effect of SP resistance on the efficacy of IPTp-SP in clearing parasites and preventing new infections. HIV-negative asymptomatic parasitemic women of all gravidity were eligible if they provided written informed consent. Recruitment is ongoing in two antenatal clinics within one hour drive south-west of Blantyre. Between December 2009 and April 2010, 79 women were successfully followed weekly until day 42, or until the day of re-occurrence of parasitemia: 46 primi- and secundi-gravidae (G1+2) and 33 multigravidae (G3+). By day 42, 34 (43.0%) experienced a re-appearance of parasitemia; This was 3, 9, and 29 by day 7 (3.3%), day 14 (11.4%), and day 28 (36.7%), respectively. Primi-, and secundi-gravidae (29/46: 63.0%) were more likely to be parasitaemic by day 42 than multigravidae (5/33: (15.2%); RR 4.16, 95% CI 1.80-9.61). Molecular analyses for SP resistance-associated mutations in dhps 436, 437, 540 and 581, dhfr 51, 59 and 164 and pfmrp1 1466, and genotyping to differentiate between recrudescent and new infections is ongoing. A study of the impact of IPTp-SP on placental malaria and birth outcome is also ongoing.

These preliminary results of the in-vivo follow-up suggest high rates of recrudescence and reinfection in primi-, and secundi-gravidae receiving IPTp with SP. This raises concern about the longevity of IPTp-SP in southern Malawi and stresses the need to explore alternative drugs to replace SP or alternative strategies to replace IPTp.

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ALANYL-GLUTAMINE PREVENTS SMALL INTESTINAL EPITHELIAL APOPTOSIS *IN VITRO* AND IN A MURINE MODEL OF WEANLING MALNUTRITION

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Malnutrition contributes to over half of all child deaths in developing countries and is associated with an enteropathy characterized by villous atrophy and increased gut permeability. Ala-Gln, a stable glutamine

dipeptide, has recently been shown to enhance catch-up growth and gut integrity in underweight children from Northeast Brazil. We sought to test the hypothesis that Ala-Gln mediates these effects via anti-apoptotic mechanisms *in vitro* and *in vivo.* Colorimetric viability assays were performed in mouse small intestine epithelial (MSIE) cells in the presence of varying concentrations of Ala-Gln. Apoptosis was assessed by annexin and 7-AAD staining and flow cytometry. To determine Ala-Gln's *in vivo* effects, we randomized dams of 10-day old C57/B6 mice to standard chow or an isocaloric, Northeast Brazil "regional" diet (low protein, low fat). On day of life 21, pups were weaned to their dam's diet and randomized to Ala-Gln solution or plain drinking water. At 6 weeks of age, mice were sacrificed to obtain jejunal specimens for morphological, immunohistochemical, and Ussing chamber analyses. Ala-Gln promoted MSIE viability in a dose-response manner and reduced early apoptosis. Pups of dams that received the regional diet exhibited failure to thrive and villous blunting, as well as decreased epithelial proliferation and increased epithelial apoptosis (as measured by BrdU and caspase-3 staining, respectively). Despite no differences in catch-up growth, undernourished pups randomized to Ala-Gln showed significant improvements in villous height and epithelial proliferation/apoptosis. In conclusion, the regional diet induces failure to thrive and environmental enteropathy-like changes in weanling mice. Ala-Gln promotes enterocyte survival and normal villous architecture in the setting of malnutrition, independent of changes in weight. Further studies are needed to define the signaling pathways by which Ala-Gln mediates these cellular responses, which are critical to recovery from the reciprocal cycle of diarrhea and malnutrition.

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POLYMORPHISMS IN INFLAMMATORY MEDIATOR GENES CONFER PROTECTION FROM SYSTEMIC BACTERIAL INFECTIONS IN *PLASMODIUM FALCIPARUM*-INFECTED CHILDREN

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Malaria and bacteremia both cause significant morbidity and mortality in Kenyan children living in holoendemic *P. falciparum* regions. We have recently reported decreased parasitemia levels in these co-infected children without exacerbation of anemia, despite elevated levels in a number of pro- and anti-inflammatory mediators in co-infected children. To further explore these findings from a genetic perspective, we examined the following single nucleotide polymorphisms (SNPs) in IL-12 (T-1188C), TNF-alpha (G-238A, C-308T, A-376T, T-1031C), IFN-gamma (receptor G-56A, G+2200A), IL-4 (C-589T, C-1335T), and IL-10 (C-592A, C-819T, T-1082C) in relation to infection status (malaria mono-infected, *Pf*[+], n=294; Gram[-] bacteremia and malaria co-infected, G[-]/*Pf*[+], n=17; and Gram[+] bacteremia and malaria co-infected, G[+]/*Pf*[+], n=9). Pearson's chi-square analysis identified associations between infection status and IFN-gammaR G-56A (*P*=0.071), IFN-gamma G+2200A (*P*=0.033), TNF-alpha C-308T (*P*=0.009), and IL-4 C-589T (*P*=0.024). Multinomial logistic regression analyses of these four SNPs, with infection status as the dependent variable (0, *Pf*[+]; 1, Bacteremia/*Pf*[+] co-infected), and sickle cell status, HIV status, G6PD status, gender, and age as covariates, revealed significant protection from bacteremia in malaria parasitemic children for IFN-gammaR G-56A [AA, odds ratio (OR)=0.394, *P*=0.029], TNF-alpha C-308T [TT, OR=0.422, *P*=0.037], and IL-4 C-589T [CT, OR=0.455, *P*=0.034]. Another set of regression analyses using high-density parasitemia (HDP, >10,000 parasites/μL) as the dependent outcome variable, demonstrated that heterozygosity at the IFN-gammaR G-56A locus was associated with increased susceptibility to HDP (GA, OR=1.607, *P*=0.050). Thus, homozygous A alleles at IFN-gammaR -56 are associated with protection from bacteremia in children with malaria, while heterozygosity at the same locus predisposes children to HDP. Taken together, these findings suggest that many of the SNPs important in malaria disease severity also play a significant role in acquisition of systemic bacterial infections in Kenyan children.

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RISK FACTORS OF *STREPTOCOCCUS SUIS* INFECTION IN VIETNAM: A CASE-CONTROL STUDY

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Streptococcus suis, an emerging zoonotic infection, is the most common bacterial cause of adult bacterial meningitis in Vietnam. The explosive outbreak of *S.suis* infection in China in 2005 with hundreds of human cases and 39 deaths and emergence across South East Asia makes this an increasingly public health problem. We conducted a case-control study to identify the risk factors of *S. suis* infection in Vietnam. A standard case-control study with appropriate hospital and matched community controls for each patient. The study was conducted between May 2006 and June 2009 at Hospital for Tropical diseases in Ho Chi Minh City, Vietnam. Patients were confirmed by blood culture or cerebrospinal fluid (CSF) culture or real-time PCR. Risk factors were assessed by a standard questionnaire, including socio-demographic and cultural characteristics, medical history, and assessment of potential risk factors. We investigated whether the bacterial is carried by patients and healthy individuals using real-time PCR and culture of throat and rectal swab samples. We recruited 101 cases *S. suis* meningitis, 303 hospital controls and 300 community controls. By multivariate analysis, we found that the risk factors of *S. suis* infection included occupations related to pigs (OR1=3.83; 95%CI=[1.33-11.04] and OR2=5.56; 95%CI=[1.51-20.57]), exposures to pigs or pork in the previous 2 weeks with skin injuries (OR1=7.29; 95%CI=[1.92-27.64] and OR2=15.76; 95%CI=[2.94-84.45]) and eating "high risk" dishes in the last 2 weeks (OR1=2.32; 95%CI=[1.21-4.46] and OR2=4.67; 95%CI=[2.26-9.62]). *S. suis* DNA was detected in rectal and throat swabs of 7 patients and was cultured from 2 of these samples, but was not detected in such samples of 1522 healthy individuals or patients without *S. suis* infection. *S. suis* is an important and emerging public health issue in Asia and one with the potential for both endemic transmission and for explosive epidemics. This case control study, the largest prospective epidemiological assessment of this disease, has identified the most important risk factors associated with *S. suis* bacterial meningitis to be occupational exposure to pigs and pig products, preparation of pork and eating 'high risk' dishes popular in parts of Asia.

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HANSEN'S DISEASE (LEPROSY) AMONG UNITED STATES-RESIDING MICRONESIANS AND MARSHALLESE

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From 2004-2008, 13% of Hansen's Disease cases reported in the United States occurred among migrants from the Federated States of Micronesia and the Republic of the Marshall Islands, countries with high HD prevalence of 10-20/10,000. Citizens of these countries (former U.S. Trust Territory of the Pacific Islands) may freely enter and work in the US, not subject to immigration restrictions. Due to economic and climatologic factors, migration is increasing; ~2400 Marshallese/Micronesians move to the U.S. annually. This study included consolidation of data from various published reports of HD indicators in the countries of origin, analysis of 1990-2009 National Hansen's Disease Program surveillance and clinical data, and collection/analysis of qualitative data relevant to disease control issues among US-resident clusters. HD prevalence and case detection rates in the two source countries remain the highest in the world, with

fluctuations due to program activity, but with little progress toward the WHO leprosy elimination target of <1/10,000. Local community rates as high as 5% have been reported. 55% of US-resident Micronesian/ Marshallese cases occurred in Hawaii. Among 74 US mainland cases in 26 states, 50% were diagnosed within 3 years of US entry, 63% did not complete treatment, and 80% had at least 1 complication, including advanced neuropathic disease. Since 1996, 17 cases have been reported in a single community in Arkansas, primarily young adult males with lepromatous disease. Comparison with rates in the Marshall Islands and in Hawaii suggests that fewer than half of cases in this community have been identified, with women, children, and tuberculoid disease under-represented. Cultural, socio-economic, and health-system barriers to HD care in this community are identified. With the goal of decreasing health disparity and preventing disability, case-finding and case-management interventions are needed in US-resident Marshallese/Micronesian communities as well as in the countries of origin.

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POLYMORPHISMS ILE655VAL IN ERBB2/HER-2/NEU RECEPTOR IS ASSOCIATED WITH HANSEN'S DISEASE (LEPROSY) IN A BRAZILIAN POPULATION

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Leprosy is an infectious disease caused by *Mycobacterium leprae* which can lead to severe permanent disability. *M. leprae* induces nervous degeneration by linkage to Schwann cell in periphery nervous system in part resulting from the interaction of the ErbB2 receptor and *M. leprae*. The objective of this study was to evaluate whether the polymorphisms in *ERBB2* gene is associated with leprosy. A total of 216 leprosy patients and 226 controls were genotyped for six markers located in *ERBB2* gene. The markers were rs2517955 and rs2517956 in the promoter region; rs1810132 and rs2952156 in intron regions, and rs1801200 and rs1058808 in exons of the *ERBB2* gene. Statistical analysis was performed by using Web-based tool Snpstats (http://bioinfo.iconcologia.net/index.php?module=Snpstats). Two SNPs were associated with Hansen's disease, respectively, SNP rs1801200 (p = 0.036, OR = 1.61, CI = 0.90 - 2.88) and the SNP rs2517956 (p = 0.047, OR = 1.53, CI = 0.84 - 2.76). The base change from A to G in the allele marker rs1801200 results in amino acid change in position 655 from isoleucine to valine. This amino acid is located in the transmembrane domain of the ErbB2 receptor, which is involved in the dimerization of ErbB2 monomers and its activation. The presence of valine induces a tighter linkage of ErbB2 monomers than isoleucine does, and results in greater stability and increased catalytic activity of ErbB2. In conclusion, presence of the polymorphic alleles in the markers rs1801200 and rs2517956 in *ERBB2* gene was found to be associated with Hansen's disease. Presence of Val in position 655 (rs1801200) results in greater stability of the two ErbB2 monomers and might increases its catalytic activity.

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A QUICK AND COST-EFFECTIVE METHOD FOR THE DIAGNOSIS OF *MYCOBACTERIUM ULCERANS* DISEASE

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Mycobacterium ulcerans causes the painless necrotizing skin disease Buruli ulcer, highly endemic in rural West and Central Africa. The mode of transmission remains unknown largely due to the lack of early case diagnosis and late treatment seeking behavior of infected individuals. Diagnosis is based on clinical presentation and microscopy followed by PCR confirmation in reference laboratories. Complete diagnosis is therefore usually late, inefficient, time consuming and very expensive. Early diagnosis is important for effective treatment to prevent the morbid effects of the disease on affected individuals. We report the development of a simple and inexpensive test that could be used in poorly to medium resourced settings at point of care facilities based on the loop mediated isothermal amplification (LAMP) method. Four sets of primers, targeting various sections of the *M. ulcerans* genome, were designed for the reaction and the assay was developed and tested on five *M. ulcerans* strains from patients in Ghana and two American Type Culture Control (ATCC) reference isolates; Ghana #970321 (D19F9) and Benin #990826 (D27D14). To determine specificity, the assay was tested on the closely related *M. marinum* 1218 and other mycolactone producing mycobacteria; *M. marinum* DL240490*, M. liflandii* and *M. pseudoshotsii*. The assay was finally tested on DNA obtained from biopsy samples from infected laboratory animals, prepared using either boil preparation or Qiagen kit extraction methods. The test was successful for DNA obtained by both methods although the latter provided the best results. Our results revealed a high specificity of the LAMP assay for selectively detecting *M. ulcerans.* Compared to the conventional PCR, the new assay is cheaper and simpler and does not require the use of a thermal cycler or electrophoresis. Results are obtained within one and a half hours and visually observed under UV light. These observations indicate that the BU-LAMP assay is suitable for early disease diagnosis and application in low resource health facilities.

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LANDSCAPE AND ENVIRONMENTAL INFLUENCES ON PRESENCE/ABSENCE OF *MYCOBACTERIUM ULCERANS* IN GHANA

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Buruli ulcer is a neglected tropical skin disease caused by *Mycobacterium ulcerans* (MU) that is highly endemic in West Africa. While the mode of transmission is unknown, many epidemiological studies have found Buruli ulcer to be associated with different types of exposure to water sources. Sixty-eight aquatic sites used for daily domestic purposes by communities in the greater Accra and Ashanti regions of Ghana were sampled in 2005-2007 to test for the presence of *M. ulcerans*. We explored the spatial distribution of the MU positive and negative water bodies as well as identified site, water, land cover, and landscape characteristics associated with MU presence using logistic regression with model selection by AIC. Kulldorff's Bernoulli spatial scan statistic using circular windows found no significant local clusters of MU positive sites, and Ripley's K function showed no significant global clustering of MU positive sites compared to negative sites. The best fitting logistic regression model identified region, elevation, region by elevation interaction, presence of urban land cover within 100m, presence of forest land cover within 1km, water hardness, and region by water hardness interaction to be associated with the presence of MU. An empirical semivariogram of the residuals from the final model revealed no significant residual spatial autocorrelation. These results support the notion that MU is an environmental organism that exists in specific niches, but whose distribution in nature may not necessarily reflect the distribution of the disease. Understanding the spatial distribution of MU as well as factors associated with its presence can further research on modes of transmission as well as identify areas in need of surveillance for Buruli ulcer.

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THE SENSITIVITY OF STANDARD CIRCULATING FILARIAL ANTIGEN TESTS AND ULTRASONOGRAPHY FOR INDIVIDUAL DIAGNOSTICS AND EPIDEMIOLOGICAL SURVEILLANCE OF BANCROFTIAN FILARIASIS

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Significant advances were made in the diagnosis of lymphatic filariasis (LF) in the past years using two tests for the detection of circulating filarial antigen (CFA) in individuals with LF: the Og4C3-ELISA (TropBio®) and the immunochromatographic test (ICT; NOW® Filariasis). These tests have been mainly used in microfilariae (Mf) carriers and both resulted in high sensitivity. To verify parasitic infection also in amicrofilaremic individuals, ultrasonography (USG) of the scrotal area is frequently used. In this study Mf-load and CFA-status (Og4C3) were assessed in healthy adult volunteers (n=1976), 535 samples were additionally analysed with ICT. All men (n=1132) underwent ultrasound examination of the scrotum. Altogether 324 were Mf+ and 1652 were Mf-. Both tests, Og4C3 and ICT, showed a high sensitivity for detection of CFA in the Mf+ samples (99% and 100% respectively) but there was a significant difference between both tests regarding the Mf- samples (consistency only in 410/483 (85%) cases). USG revealed that 201 men were FDS+/Mf+, 151 FDS+/Mf-, 74 FDS-/Mf+ and 706 FDS-/Mf-. The sensitivity of Og4C3 and ICT was high in microfilaremic patients (99% or 100%). The sensitivity of the Og4C3 for FDS+/Mf- men was 91%, that of the ICT 82%. There was a significant difference between both tests in the assessment of the FDS-/Mf- patients (consistency in 113/140 (81%)). In 74/275 (27%) Mf+ men, life adult worms could not be detected by USG. In conclusion, confirmative to a former trial, in 73% of the Mf+ individuals life adult worms were detected by USG. The lower detection of the USG is presumably caused by adult worms located in sites of the body other than the scrotum. Og4C3 and ICT both show a high and comparable sensitivity in the detection of Mf+ individuals while in FDS+/Mf- cases the sensitivity of antigen detection is lower. Particularly in absence of Mf and FDS, Og4C3 and ICT show a lack of consistency. Therefore antigen results from Mf- individuals should be interpreted taking this caveat into account.

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IDENTIFICATION OF A *WUCHERERIA BANCROFTI* LARVAL STAGE SPECIFIC STAGE PROTEIN THAT IS BOTH SENSITIVE AND SPECIFIC IN DETECTING ANTIBODIES IN *W. BANCROFTI* INFECTED PATIENTS

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The current antibody tests used for mapping the distribution of lymphatic filariasis (LF) and for monitoring progress in elimination programs suffer from poor specificity because of considerable cross-reactivity with antibodies induced by other filarial infections such as *Loa loa*, *Onchocerca volvulus*, and *Mansonella* spp. Using the dCAS bioinformatics package, we assembled 2048 expressed sequence tags (EST) from the L3 infective larvae of *W. bancrofti* into non-redundant contigs which were then assessed for homology to protein and nucleotide databases as well as head-to-head against contig sets assembled from L3 larval ESTs of *B. malayi* (Bm - 5068 ESTs), *O. volvulus* (Ov - 4166 ESTs), and *Loa loa (*Ll- 3315 ESTs). Nineteen potential L3- and Wb-specific antigens were identified and expressed as fusion proteins with Renilla luciferase in mammalian cells. Screening of cell lysates by a Luciferase Immunopreciptation System (LIPS) assay revealed that only 1 of the 19 antigens (Wb-123) was both highly immunogenic and Wb-specific. Using a broad panel of well-defined sera from normal North Americans (n=53) and patients infected exclusively with Wb (n=43), Ll (n= 70), Ov (n=43), or intestinal helminths (n= 21), the Wb-123 based LIPS assay could identify sera from all of the Wb-infected individuals (MF+ or CAg+ from diverse geographic regions) with 100% sensitivity and 100% specificity compared to sera from uninfected controls and those with intestinal helminths. When specificities and sensitivities were assessed using sera from Ll-infected or Ov-infected individuals as the comparator, the sensitivities ranged between 98-100% and the specificities between 97-98%. Thus, we have identified an L3- and Wb-specific antigen that can be used not only as a rapid and specific tool to diagnose individual *Wb* infections but also as a sensitive, high-throughput, and potentially point-of care method for early detection of recrudescent infections in areas of control and for mapping new areas of Wb transmission.

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CARDIAC LESIONS IN AN AREA HYPERENDEMIC FOR LOIASIS IN CAMEROON

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Although the majority of patients with loiasis are asymptomatic despite high levels of blood microfilariae, characteristic symptoms include migratory angioedema and subconjunctival migration of the adult worm. Serious complications, including endomyocardial fibrosis (EMF), have been described; however, the prevalence of such complications in endemic areas is unknown and likely underestimated. To assess the cardiac complications related to loiasis, we performed a cross-sectional, study of 297 adult (>15 years of age) residents of a hyperendemic focus of loiasis in Cameroon. Subjects with evidence of onchocerciasis or lymphatic filariasis, a history of cardiovascular disease prior to their settlement in the study area or any antifilarial treatment taken during the last two years were excluded from the study. All subjects underwent a detailed clinical examination, assessment of microfilaremia by calibrated thick smear of daytime blood, *Loa loa* serology (SXP LIPS), and echocardiography performed by an experienced cardiologist. Of the 297 subjects, 180 had detectable *Loa* microfilaremia, 39 had both *Loa* and *Mansonella perstans* microfilaremia and 63 had no serologic or parasitologic evidence of *Loa* infection. Echocardiography was abnormal in a high percentage (84.5%) of patients and included valvular or endocardial calcifications (70%), diastolic dysfunction (35.7%), cavity dilatation (34.3%), valvular insufficiency (18.5%), left ventricular hypertrophy (9.8%), pericardial lesions (2.4%) and EMF (1.01%). Although the frequency and distribution of these abnormalities was not statistically different between subjects with and without loiasis, the number of uninfected subjects was small. Of note, all 3 subjects with EMF had detectable *Loa* infection, negative stool examination for intestinal helminths and marked eosinophilia. Although these data are consistent with an increased prevalence of cardiac

abnormalities, including EMF, in an area hyperendemic for loiasis, the role of loiasis in the pathogenesis of these abnormalities remains to be elucidated.

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TARGETING *WOLBACHIA* ENDOSYMBIONTS IN *ONCHOCERCA VOLVULUS* EFFECTIVELY CLEARS PERSISTENT MICROFILARIAE IN THE SKIN OF ONCHOCERCIASIS PATIENTS IN WHOM REPEATED IVERMECTIN TREATMENT HAD FAILED TO CLEAR

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Ivermectin (IVM) has been the drug of choice for the treatment of onchocerciasis since 1987. However, there have been reports of persistent microfilariae (Mf) in the skin of some people after many rounds of IVM treatment in some districts in Ghana. These indications are consistent with the emergence of drug resistance or sub-optimal response to IVM. To assess the effect of targeting Wolbachia endosymbionts in O. volvulus on onchocerciasis patients in whom repeated IVM treatment had failed to mediate Mf clearance, 149 patients were recruited in 2 districts in Ghana where IVM resistance has been reported. They were treated with either 100mg/d doxycycline (Doxy) or matching placebo for 6 weeks. Three and 12 months after Doxy treatment, all patients took part in ongoing IVM mass treatment. Patients were snipped before, 12 and 20 months after treatment to assess the levels of Mf that IVM could not clear. Entomological work was also carried out in all the studied villages before and after Doxy treatment.

Before treatment, of the 73 patients allocated for doxycycline, 66% had persistent Mf in the skin and 34% had only nodules but no skin Mf, and of the 76 patients allocated for placebo, 63% had persistent Mf in the skin and 37% had only nodules (P=0.74). However, at 12 months after Doxy treatment, of the 72 Doxy-treated patients snipped, 10% still had low numbers of Mf in the skin and 90% had no Mf at all. Of the 71 placebo patients snipped, 58% still had Mf in the skin while 42% had no Mf (P<0.001). At 20 months post therapy, only 3% of the 69 Doxy patients had low Mf and 97% were Mf negative. In contrast, of the 71 placebo patients, 69% still had Mf while only 31% had no Mf. This difference between the Doxy and placebo groups was significant (P<0.001). Doxy cleared Wolbachia significantly compared to placebo group and shows embryostatic effect in the adult worms compared to placebo patients. A comparison between pre-treatment and post treatment transmission parameters indicated a significant reduction after intervention in most areas. Doxycycline clears Wolbachia from O. volvulus worms, and resulted in embryogenesis blockade. Therefore, targeting Wolbachia in O. volvulus is effective in clearing Mf in the skin of onchocerciasis patients in whom repeated standard treatment has failed to clear; thus strategies may be developed including anti-Wolbachia l treatment to control the re-emergence of onchocerciasis in areas where infections persist despite the use of IVM.

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HIGH DOSE BIANNUAL ALBENDAZOLE AND IVERMECTIN SUPPRESS *WUCHERERIA BANCROFTI* MICROFILARIAL LEVELS MORE EFFECTIVELY THAN STANDARD DOSE ANNUAL TREATMENT

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Annual mass treatment with albendazole and ivermectin is the mainstay of current strategies to interrupt transmission of *Wuchereria bancrofti* (Wb) in Africa. More effective microfilarial suppression could reduce the time necessary to interrupt transmission and ease the economic burden of such programs in countries with limited resources. To determine the effect of increased dose and frequency of albendazole/ivermectin (A/I) treatment on microfilarial (mf) clearance, 40 Wb microfilaremic residents of an endemic area in Mali were randomized to receive three doses of standard annual A/I therapy (400 mg/150 mcg/kg; n=21) or six doses of twice-yearly increased dose A/I therapy (800 mg/400 mcg/kg; n=19). Mf levels were assessed by Nuclepore filtration of 1 ml of blood and circulating antigen (CAg) levels by TropBio™ ELISA. We have previously reported increased efficacy of twice-yearly high dose treatment in reducing mf counts at 12, 18 and 24 months as compared to standard dose annual therapy with no mf detected in subjects in the twice-yearly group at any time point after 6 months. At 30 months, only 1/17 subjects in the annual group and 0/17 subjects in the twice-yearly group had detectable mf (p=NS). As at prior time points, a significant and comparable decrease in CAg levels was seen in the annual and twice-yearly treatment groups at 30 months with geometric mean (GM) % pre-treatment levels of 74% and 54%, respectively. Thirty-six month followup is planned for July 2010. These findings suggest that increasing the dose and frequency of A/I treatment leads to more rapid suppression of microfilaremia than standard annual therapy and that this effect is not due to an enhanced adulticidal effect. Consequently, twice-yearly high dose treatment is likely to have the greatest benefit in accelerating transmission interruption in regions where mass treatment has been non-existent or suboptimal. Additional studies examining the independent effects of dose and frequency are clearly needed.

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A HYDROCELECTOMY PROGRAM FOR LYMPHATIC FILARIASIS IN LÉOGANE, HAITI: CLINICAL INFORMATION AND SURGICAL OUTCOMES

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Lymphatic filariasis (LF) has been endemic in Haiti for over 250 years with a current estimate of 8 million people at risk of infection. In Léogane Commune, up to 30% of adult males suffer from hydrocele, the most common manifestation of chronic LF. Since 2001, a surgical program providing hydrocelectomy has been in operation at Hôpital Sainte Croix and Hôpital Cardinal Légère in Léogane. We assessed clinical data and surgical outcomes for 491 men who underwent hydrocelectomy between 2001 and 2008. Patients ranged in age from 14-85 years (mean, 42 years) and reported an average of 5.6 years with the hydrocele (range, 3 days

- 26 years). Over this eight year period, a total of 792 hydrocelectomies were performed (bilateral hydrocelectomies were counted as two procedures) with the majority (98%) of these procedures utilizing the 'excision technique' where complete excision of the tunica vaginalis is performed. The average hydrocele volume was 510 cc (range 3-2,100 cc) and 116/390 (30%) men were positive for filarial antigen by ICT card test prior to surgery. Variability of hydrocele fluid types were noted intraoperatively including pure hydrocele (n=478) (60.4%), lymphocele (n=269) (34.0%), chylocele (n=23) (2.9%), hematochylocele (n=17) (2.1%), and hematocele (n=5) (0.6%). Only 32/491 (6.5%) men had a negative outcome following surgery, defined as hydrocele recurrence, development of a new hydrocele, post-operative infection, or hematoma formation. Potential predictors of negative clinical outcome will be highlighted and discussed. These results illustrate the clinical variability of filarial hydrocele and the success of the Léogane hydrocelectomy program.

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ECONOMIC AND PSYCHOSOCIAL IMPACT OF HYDROCELE AND THE BENEFITS OF HYDROCELECTOMY

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Hydrocele is a major public health problem in Lymphatic Filariasis (LF) endemic countries. It causes disability and negatively impacts on productivity, quality of life and sexuality. Global estimates suggest 80 countries are endemic for LF, affecting over 120 million people. In highly endemic countries, hydrocele can affect up to 20% of adult males. Globally, little quantitative data exist on the economic and psychosocial effects of hydrocele and the benefits surgery can provide. This study was undertaken in Ghana to provide greater understanding of the psychosocial and economic burden of hydrocele and to facilitate policy formulation. This was a longitudinal study, comprising of pre-surgical, surgical and a series of post surgical evaluation over a two year period. The pre-surgical phase involved identification and recruitment of respondents and confirmation of the presence of hydrocele and the surgical phase involved the provision of surgery. The post surgical phase involved evaluating the clinical outcome of surgery and assessing the economic and psychosocial effects of surgery at predetermined timelines. A modified time series evaluation scheme, where patients are assessed at six points: before surgery, and 3, 6, 12, 18 and 24 months after surgery was adopted. Data collection was done with semi-structured questionnaires and Focus Group Discussions. Of 1,201 men reporting scrotal swellings, 392 were confirmed to have hydrocele. Of these, 323 gave informed consent and were recruited into the study. Post-surgical evaluation identified significant improvement in economic situation (66.8%), performance of daily activities (95.0%), ability to work/engage in income generating activities (88.2%), family life (67.9%), sexual performance (35.2%), quality of life and self reliance among the respondents. The findings also showed significant reduction in personal health related costs as very few respondents sought medical treatment after surgery.

Filariasis predominantly affects rural communities that depend almost entirely on subsistence agriculture. Household economies are extremely fragile and unable to extend to surgery for hydrocele repair. Results of our study clearly show the economic and social benefits of hydrocelectomy and argue strongly for providing access to surgical repair in all programs targeting LF and the Neglected Tropical Diseases.

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PLASMODIUM RHOMBOID PROTEASE, ROM3, IS NECESSARY FOR DEVELOPMENT WITHIN THE MOSQUITO VECTOR

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Transmission of the malaria parasite into the mosquito vector occurs when the mosquito ingests gametocytes during a blood meal from an infected host. Within the midgut, ingested gametocytes transform into motile ookinetes that traverse the midgut epithelia and settle within the basal lamina. Nestled in this safe and nutrient-rich environment, the ookinete becomes sessile, rounds up and transforms into the oocyst. The oocyst becomes a spherical syncitium and enlarges to accommodate the thousands of nuclei which will be repartitioned to daughter sporozoites. The process of sporozoite budding within the oocyst is called sporogony. We characterized a rhomboid protease, ROM3 that is specifically expressed in the sexual stages of the malaria parasite. Using the rodent malaria model, *Plasmodium yoelii*, deletion mutants of pyROM3 were generated. We find that *pyrom3-/-* parasites progress normally through gametocytogenesis and gametogenesis leading to oocyst development, but are unable to undergo sporulation. ROM3 is necessary for the production of infectious sporozoites since *pyrom3-/-* parasites are unable to transmit disease to mice. Ultrastructural analysis of *pyrom3-/-* mutant oocysts reveals a defect in the early stages of sporulation prior to sporoblast formation and membrane retraction. The defect is characterized by accumulation of membranous whorls, unusually enlarged nuclei, and an inability to initiate membrane retraction for the onset of sporulation. This study provides novel insights into the function of a rhomboid protease in *Plasmodium* parasites during intracellular development. Further studies into how ROM3 exerts its function for the proper maturation and onset of sporulation should be helpful in the identification of potential substrates whose processing is required for transmission of malaria parasites.

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3D ANALYSIS REVEALS COUPLED DYNAMICS OF CHROMATIN AND NUCLEAR PORES IN THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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The deadliest form of human malaria is caused by the protozoan parasite *Plasmodium falciparum*, which is believed to be responsible for millions of death cases each year. The parasite virulence is attributed to its ability to modify the infected erythrocyte by means of antigenic variation, in order to evade immune attack and maintain long-term chronic infections. The complex life cycle of malaria parasites is associated with tight transcriptional regulation of gene expression. Nuclear positioning may play an important role in the regulation of *P. falciparum* virulence genes. In order to investigate the nuclear dynamics in *P. falciparum* we have applied an emerging technique of electron microscopy that provides automated acquisition of serial section images as thin as 10 nm. Thus a single nucleus is spanned typically by 150 sections, allowing direct generation of a 3D model without requiring tomographic reconstruction. We generated 3D models of the parasite nucleus at distinct stages of development within the infected red blood cell. We found dramatic changes in chromatin organization coinciding to a previously-described pattern of gene expression during the mid- to late schizont phase. We also found a clear correlation between euchromatin positioning at the nuclear

envelope and the local distribution of nuclear pores, as well as a dynamic nuclear polarity during schizogony. Our results suggest that dynamics in nuclear architecture during parasite development are correlated with gene expression.

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4D-LIFE CELL MICROSCOPY OF ASEXUAL *PLASMODIUM FALCIPARUM* DEVELOPMENT AND HOST CELL MODIFICATIONS

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The visualization of growing *Plasmodium falciparum* blood stage parasites is crucial for the understanding of dynamic cellular processes such as protein transport. However, this has not been achieved so far. We established 4D-imaging (time lapse of 3D reconstructions) of individual *P. falciparum* parasites across the entire asexual blood cycle. Our time lapse movies show an unexpectedly dynamic parasite. It provides a reference for the asexual development cycle that includes indicators for the build up and completion of host cell modifications, onset of feeding and active preparation for egress. Using 4D-imaging we analysed parasite-induced host cell modifications termed Maurer's clefts, structures important for the export of parasite proteins. Our data show that the Maurer's clefts pass through three distinct phases during parasite maturation. Further we show that Maurer's clefts are present much earlier in the cycle than generally thought and that no clefts are generated thereafter. This contradicts the widespread view that there is continued formation of clefts from the parasitophorous vacuole membrane surrounding the parasite. Consequently we find no indication for the proposed protein export via nascent clefts but show that different membrane associated proteins reach already formed clefts located in the host cell cytoplasm. Thus, in addition to providing a new view of the asexual blood development, our work challenges generally held views regarding the formation of Maurer's clefts and protein export in malaria parasites.

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SYSTEMIC AND LOCAL CONTROL OF DENDRITIC CELL (DC) POPULATIONS DURING GUT-DWELLING HELMINTH INFECTION: INSIGHTS INTO LOCAL VERSUS BYSTANDER EFFECTS OF CHRONIC INFECTIONS

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Parasitic worms (helminths) have co-evolved alongside their host's immune system to establish long-term infections. Understanding the immunological basis of these interactions is important given the strong correlations between presence of infection and diminished local (e.g. Crohn's disease), and systemic, allergic or autoimmune conditions; known as the "hygiene hypothesis". Regulatory T-cells. (Treg) play a key role in mediating protection, however, the contribution of other immune cell types, particularly antigen presenting cells (APC), remains unresolved. Using a murine model of chronic gut helminth infection, *Heligmosomoides polygyrus*, we characterize changes to local and systemic APC. We found that the most dramatically increased population in the gut are serosal macrophages. Despite this change, CD103+ lamina propria DC are maintained and the CD103+-DC to CD103--macrophage ratio is unaltered. This correlates with maintenance of Foxp3+ Treg conversion to food antigens even in the presence of Th2-cells, which are considered to be counter-regulatory to this process. Moreover, the CD103+ DC retain their ability to metabolize vitamin A to retinoic acid in the altered environment providing one explanation for the maintenance of Treg conversion. Systemic changes to CD103 expression are also evident; in the spleen CD103 is upregulated on the CD8α+ DC. These data add to our understanding of how APC are altered during establishment of a chronic infection, and suggest that changes in DC populations and function can extend beyond the primary site of parasitism. This provides a previously unrecognized mechanism by which APC-mediated bystander suppression may contribute to inhibition of allergic and autoimmune reactivity.

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LARVAL EXCRETORY / SECRETORY PRODUCTS OF THE HELMINTH *TRICHURIS SUIS* MODULATE THE ONSET OF INFLAMMATORY DISEASES

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Infections with parasitic helminths are highly prevalent in the developing world and are of particular importance as they have the ability to complicate vaccination and drug efficacy in endemic areas due to their potent immunomodulatory capacity. In recent years epidemiological studies have identified a strong correlation between loss of helminth infections in the western world and the development of autoimmune and inflammatory diseases - leading to a revision of the hygiene hypothesis to include helminths as a critical regulator of immune homeostasis. The ability of helminth-derived molecules to manipulate the immune system and interfere with the development of unrelated diseases thus provides the potential for development of new treatment strategies.

Utilising first stage larvae of the porcine helminth Trichuris suis we isolated potent parasite derived products, which demonstrated an immunodulatory capacity in vitro. Furthermore, treatment with the T. suis products in a mouse model of airway hyperreactivity led to a significant reduction of disease parameters including reduced airway eosinophilia and lymphocyte infiltration, suppressed antigen specific cytokine responses and reduced antigen specific IgE. Similarly T. suis products were effective in the suppression of the development of Experimental Autoimmune Encephalomyelitis (EAE). Thus, products of Trichuris suis represent promising candidates for the development of drugs based on helminthic therapy.

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GUT MICROFLORA REGULATES GUT MACROFAUNA: EXPLOITATION OF THE INTESTINAL ECOSYSTEM BY A PARASITIC NEMATODE

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Trichuris muris lives in close association with the host, embedding into the caecal epithelium, burrowing through cells and forming syncitial tunnels. We demonstrate for the first time that the *T. muris* life cycle is also intricately linked to the host gut microflora. *T. muris* eggs can be induced to hatch naturally *in vitro* by culturing with bacteria. We have identified the surface protein on gram negative bacteria that can facilitate this hatching. Using genetically modified *E. coli,* Fim H, an adhesin present on type I fimbrae was shown be of major importance. In order to ascertain whether these types of interactions are required *in vivo*, broad spectrum antibiotic treated mice were infected with *T. muris*. We found that antibiotic treatment substantially reduced the number of bacteria in the gut and significantly reduced the establishment of these worms, indicating an effect on hatching. Furthermore, treatment also influenced the normal infection induced immune response mounted by the host, skewing towards a Th2 phenotype. Increased levels of IL-4 and IL-13 and reduced levels of pro inflammatory cytokines and IL-17 were observed. Critical interactions between bacteria (microflora), parasite (macrofauna) and

the host introduce a new and important dynamic to the intestinal niche which has fundamental implications for our current concepts of intestinal homeostasis and regulation of immunity.

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HOST IMMUNE RESPONSES TO INFECTION WITH LEISHMANIA GUYANENSIS

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Infections with *L.guyanensis* are distinguished by their ability to disseminate from the initial site of infection to the nasopharyngeal tissues forming destructive secondary lesions. Mucocutaneous Leishmaniasis (MCL) patients have hyper-inflammatory responses with a high degree macrophage and T cell infiltration into the lesion. We show that hamster derived metastatic (M+) or patient derived MCL *L.guyanensis* parasites induce elevated levels of chemokines and cytokines in infected BMMf, namely CXCL10, CCL5, TNF-α, IL-6, and IFN-β as compared to BMMf infected with M-, or (Cutaneous Leishmaniasis) CL or *L. major* LV39 parasites. The induction of these cytokines and chemokines by M+ parasites was completely abrogated in infected TLR3-/- BMMf. *L. guyanensis* parasites can be infected with *Leishmaniavirus* (LRV1) and we detected the 5.3Kb dsRNA genome of LRV1 by gel electrophoresis in M+ or MCL promastigotes and showed by PCR with that M+ and MCL promastigotes have a higher viral load (LRVhigh) than M- or CL (LRVlow). Purified LRV1 dsRNA stimulated increased cytokine and chemokine transcripts in C57BL/6 BMMf, which was significantly diminished in TLR3-/- BMMf. Finally, we show by *in vivo* infection experiments that TLR3-/- mice were more resistant to infection with LRV1high M+ parasites than C57BL/6 mice as shown by a decreased footpad swelling peak associated with a lower parasitemia, whilst there were no observable difference in disease evolution in LRV1low M- parasites. This study shows that there is a correlation between the disseminating capability of parasites, presence of high LRV1 burden, and the induction of inflammation in the mammalian host. Could the presence of high LRV1 viral burden be involved in MCL development in humans?

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MULTIPLE ARGININE METHYLTRANFERASES AND METHYLPROTEINS IN THE PARASITIC PROTOZOAN *TRYPANOSOMA BRUCEI*

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Arginine methylation is a common posttranslational modification catalyzed by a family of enzymes termed protein arginine methyltransferases (PRMTs). In kinetoplastid parasites, gene expression is modulated post-transcriptionally by RNA stabilization, translation, and mitochondrial RNA editing. These processes rely on a large number of RNA binding proteins (RBPs), a class of proteins that are commonly methylated on arginine (arg) residues in yeast and mammals, suggesting a prominent role for PRMTs in kinetoplastids. Kinetoplastids stand out among single celled eukaryotes in that their genomes encode five putative PRMTs, a relatively large number. *In vitro* studies reveal both homologues of yeast and mammalian PRMTs, as well a novel, extraordinarily active Type III PRMT. *In vivo* RNAi studies in *T. brucei* highlight the Type I TbPRMT6 as an essential PRMT with a role in cytokinesis. Other PRMT knockdowns fail to exhibit a growth phenotype in PF, and studies are ongoing in BF. Lack of a growth phenotype may reflect redundancy between PRMTs, as observed in other organisms. We are currently investigating redundancy and cooperation between PRMTs by simultaneous RNAi studies. Regarding PRMT substrates, immunoblotting with anti-methylarg antibodies reveals arg methylproteins enriched in the cytosol, nucleus, and mitochondrion of *T. brucei*. Currently, we are employing combined ETD/CID mass spectrometry to define the *T. brucei* arg methylproteome, and we have already identified methylproteins from many functional classes of proteins. *In vivo* studies are also underway involving the essential RNA editing factor TbRGG2, which is extensively methylated *in vitro* and *in vivo* to determine the role of methylation in its function. Together, these studies will provide insight into the role of arg methylation in kinetoplastid gene regulation.

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CELLULAR LOCALIZATION AND FUNCTIONAL CHARACTERIZATION OF A VOLTAGE-DEPENDENT POTASSIUM CHANNEL IN *TRYPANOSOMA CRUZI*

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Ion homeostasis is a dynamic mechanism that mediates adaptation to environmental and intracellular variations. In *Trypanosoma cruzi*, changes in potassium equilibrium seem to be involved in plasma membrane potential regulation, pH homeostasis, and osmotic balance. We identified, cloned and expressed a *T. cruzi* gene (Tc00.1047053511301.140) encoding a voltage-dependent potassium channel (TcKv). The predicted structure possesses a tetramerization domain and two transmembrane domains, characteristic of inwardrectifier potassium channels. TcKv is expressed in the three life cycle stages of the parasite, with a slightly different subcellular localization, being flagellum-related in trypomastigotes. When expressed in mutant yeast, TcKv restored the normal phenotype, suggesting its function as a K+ permeability pathway. To further characterize this channel the His-tagged protein was expressed in *E. coli*, purified and fused with liposomes in a reconstituted system. Using patchclamp technique we established that TcKv behaves as an inwardrectifier channel, with conductances of 54pS and 112pS at +80 and -80 mV, respectively. The selectivity sequence for monovalent cations is K>Cs>NH4>Rb>Na>Li. The relative permeability ratio K+/Na+ is about 3 and K+/Cl–is close to five, indicating a weak selectivity filter. Permeability for divalent cations was low, showing strong blockage by Ba2+ and Ca2+. Interestingly, when parasites are exposed to hyperosmotic conditions, TcKv localization changes. In epimastigotes it translocates to the plasma membrane, whereas in trypomastigotes is released to the medium, suggesting a differential role of TcKv in *T.cruzi* osmotic response of different stages. In conclusion, we have developed a reliable strategy to characterize ion channels in trypanosomatids, which could contribute to elucidate their physiological roles. [This work was funded in part by the American Heart Association and the NIH]

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THE SECRETED PSEUDOKINASE, ROP5, IS CRITICAL TO *TOXOPLASMA* PATHOGENESIS

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Toxoplasma gondii secretes a variety of effector proteins into its host cell cytoplasm during invasion. We have identified one of these factors, the pseudokinase ROP5, as absolutely critical to pathogenesis in mice; while ablation of the ROP5 locus (ΔROP5) causes no phenotype to *Toxoplasma* growth *in vitro*, ΔROP5 parasites are completely unable to cause pathology in a mouse. Furthermore, allelic variation of ROP5 is responsible for a >104 difference in virulence (LD50) in a mouse model of disease. ROP5 appears to exert its profound effect on disease outcome through subversion of the innate immune system; ΔROP5 parasites elicit a significantly stronger pro-inflammatory response during early infection than do wild-type parasites, and appear to be cleared within 10 days post-infection. We

have solved the crystal structure of the pseudokinase domain of ROP5 and have found that polymorphisms in ROP5 between the avirulent and virulent alleles cluster almost exclusively in the substrate binding regions and former active site of the kinase domain, which we have verified as catalytically inactive, though it still maintains its ability to bind ATP. This strongly suggests that differences in virulence are mediated by differences in interaction of binding partners with ROP5's pseudokinase domain. We hypothesize that ROP5 is acting to dysregulate one or more host signaling networks, thereby influencing the outcome of disease. To address this question, we are using phospho-flow cytometry to compare the cellular activation states of the immune systems of mice infected with either wild-type or ΔROP5 parasites. In addition, we are interrogating ROP5's binding partners by co-immunoprecipitation and mass spectrometry.

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