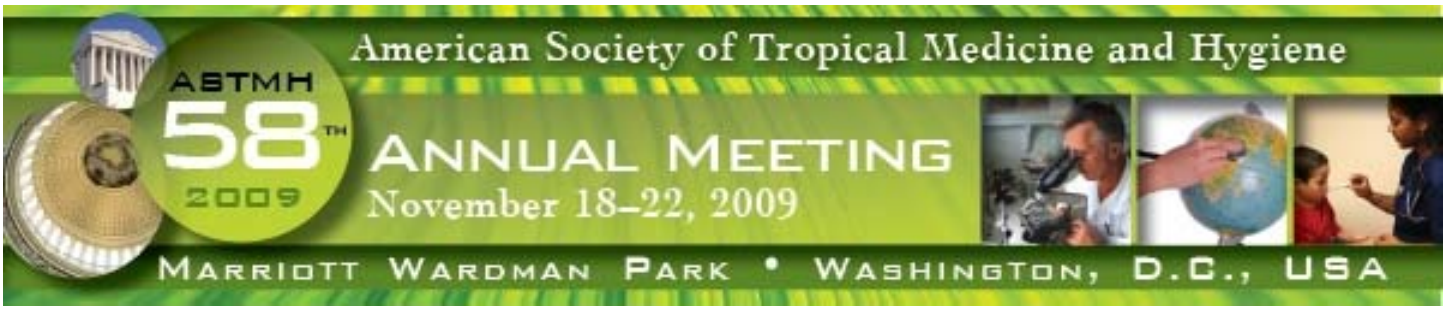

Late Breaker Abstracts

2480

CLONING AND CHARACTERIZATION OF *SCHISTOSOMA JAPONICUM* INSULIN RECEPTORS: POTENTIAL NEW INTERVENTION TARGETS AGAINST SCHISTOSOMIASIS

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Adult schistosomes depend for growth and development on hormonal signals from the mammalian host, which may include the insulin signalling pathway. To determine the precise role of insulin receptors in schistosome biology, we isolated two types of insulin receptors from *Schistosoma japonicum, S. japonicum* insulin receptor 1 (SjIR1) and SjIR2, with features similar to insulin receptors from other taxa. The sequences share 70% and 74% sequence identity to *S. mansoni* insulin receptor 1 and 2 (SmIR1 and SmIR2), respectively. SjIR1 and SjIR2 are conserved in tyrosine kinase domain to the other IRs, such as humans, mouse and *Drosophila melanogaster*. Phylogenetic analysis showed that SjIR2 and SmIR2 are close to *Echinococcus multilocularis* insulin receptor (EmIR), which is only one insulin receptor isolated in the tapeworm, indicating that SjIR2, SmIR2 and EmIR may be orthologs sharing the similar roles in the both schistosomes and *Echinococcus*, while IR1 homolog in *E. multilocularis* would have been lost during the cestode evolution. Real time PCR showed that the SjIRs were differentially expressed in different stages of *S. japonisum*, mainly in the stages in mammalian host, suggesting SjIRs may be involved in the host-parasite crosstalk. Yeast two-hybrid and BIAcore analysis demonstrated that the SjIRs specifically bound human insulin both *in vivo* and *in vitro*. Immunolocalization analysis revealed that SjIR1 is located on the internal and external teguments of adult worms, whereas SjIR2 is located in the parenchyma of male and vitelline of female worm, suggesting that SjIR1 may be involved in utilizing host insulin from the environment and SjIR2 is likely involved in providing insulin to stimulate cell growth and differentiation, and also SjIR2 may play an important role in fecundity of female worm. Adult worms of *S. japonicum* possess insulin receptors that can specifically bind to insulin, indicating that the parasite can utilize host insulin for development and growth by sharing the same pathway as mammalian cells for controlling cell differentiation and proliferation. A complete understanding of the role of SjIRs in the biology of *S. japonicum* may result in their use as new targets for drug and vaccine development against schistosomiasis.

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A DNA vaccine trial using DBP, MSP-1, and AMA-1 of the reemerging Korean *Plasmodium vivax* isolates

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Studies on the reemerging Korean *Plasmodium vivax* malaria after 1993 have focused on identifying antigenic proteins and genotypes of isolates from patients. Recently, in order to evaluate the usefulness of DNA vaccine candidates using merozoite antigens, we cloned 3 major candidate antigens, i.e., DBP, MSP-1, and AMA-1. The DNA fragments of PCR products were subcloned into TA vectors and the genes encoding these antigens were sequenced. Then, the antigens were cloned into the pcDNA 3.1(-) vector, and expression plasmids were finally constructed. The vector was inserted with mutant ubiquitin genes, and thus the expression plasmids including each candidate antigen were expected to be generated by the ubiquitin-proteasome pathway in mammalian cells. COS-7 cells were transfected with the expression plasmids using lipofectamine, and the merozoite antigens were successfully expressed in cultured COS-7 cells. The molecular weight of AMA-1 appeared to be 56.8 kDa and that of DBP was 37.8 kDa. Our vectors containing the mutant ubiquitin may induce antigen presentation to MHC class I molecules. Mice were challenged by intramuscular injection with DNA vectors carrying 3 kinds of the DNA vaccine candidates. The results showed that inoculation of AMA-1-inserted pcDNA vector induced significant increases of IFN-γ mRNA signals and total IgG and IgG2a titers. Similarly, DBP- and MSP-1-inserted pcDNA vectors also induced increases of IFN-γ mRNA signals. In addition, we immunized mice using a gene gun carrying the target DNA.

We could confirm a significant increase of $CD8^+$-T cell population in the spleen as compared with controls immunized with pcDNA 3.1(-) vector alone. Our results suggest that DNA vectors carrying the DNA of the reemerging Korean *P. vivax* can induce strong immune responses in recipient mice.

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Patterns of valvular involvement in rheumatic heart disease

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Background: The incidence of acute rheumatic fever (ARF) is high in Nepal. Its sequel in the form of chronic rheumatic valvular heart disease is a major cause of mortality and morbidity among pediatric and adult populations. The aims of the study were to analyze patterns of valve involvement in the pediatric and adult age groups and their complications.
Methods: The study was a retrospective case series analysis that included 1606 patients with chronic rheumatic valvular heart disease who presented to a tertiary care center in eastern Nepal over a period of 10 years. All of these patients underwent transthoracic echocardiography (TTE) by trained physicians.
Results: Female to male ratio in the study population was 1.6: 1. The combined mitral and aortic valve lesions were the commonest pattern of rheumatic valve involvement seen in 51% of pediatric (age≤ 18 years) and 49% of adult (age >18 years) populations. It was followed by the involvement of mitral valve alone with a frequency of 44% and 47% in pediatric and adult populations respectively. Only 5% of pediatric and 4% of adult populations had aortic valve involvement as a single valve lesion. Rheumatic involvement of tricuspid and pulmonic valves was not observed in any of these cases. Pulmonary arterial hypertension was observed in 28% of pediatric age group and 40% adult age group. Echocardiographic evidence of infective endocarditis was seen in 6.5% (pediatric) and 5.9% (adult) patients. Left atrial (LA) clot was more common in adult population (2.8%) than in pediatric population (1.8%) as detected by TTE. Only 3 out of 1606 patients had undergone valve replacement surgery.
Conclusion: The pattern of valve involvement in chronic RHD in Nepal doesn't differ from other developing countries. The high frequencies of pulmonary artery hypertension, infective endocarditis and LA clot in these patients lead to increased mortality and morbidity. Appropriate early management of RHD can improve the quality of life. We need to focus on ARF prophylaxis in order to prevent chronic RHD.

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The role of nasopharyngeal load of *Streptococcus pneumonia* and its interaction as risk factors for childhood pneumonia in Vietnam

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Background: The role of nasopharyngeal bacterial load and its interaction with viral co-infection in the development of lower respiratory tract infections (LRTIs) remain unclear. We hypothesized that a high nasopharyngeal bacterial load may associate with LRTIs.
Methods: A case-control study for pediatric LRTIs was conducted in NhaTrang, central Vietnam. A total of 555 consecutively hospitalised children were identified (274 radiographic confirmed pneumonia (RCP) and 281 other LRTIs) and 350 healthy controls were randomly selected from the community. PCR-based methods were used to detect three bacteria and 13 respiratory viruses in nasopharyngeal samples. Then quantitative measurements (real-time quantitative PCRs) of three species of bacteria: *Streptococcus pneumoniae* (SP), *Hemophilus influenzae* (HI) and *Moraxella catarrhalis* (MC) were analyzed among the three groups of participants both in the presence or absence of viral co-infection.
Results: The median nasopharyngeal load of SP in RCP children was significantly higher than those in either healthy controls ($P<0.0001$) or other LRTI children ($P<0.001$). After controlling for potential confounders, high nasopharyngeal load of SP ($>=10^7$ bacteria/ml secretion) was strongly associated with RCP (adjusted odds ratio (OR), 3.26; 95% confidence interval (CI), 1.77 to 6.00). Children with viral co-infection had a 15-fold higher nasopharyngeal load of SP compared to those without viral co-infection in RCP group ($1.4x10^7$/ml versus $9.1x10^5$/ml, $p=0.0001$). This association remained strong after adjustment (adjusted OR, 6.83; 95% CI, 2.35 to 19.84).No association was found between either high bacterial load of MC or HI and viral co-infection in either RCP or other LRTI groups ($P>0.05$).
Conclusions: High nasopharyngeal load of SP was associated with RCP in Vietnamese children. Viral co-infection played an important role in increasing nasopharyngeal bacterial load of SP but not HI and MC. Thus, vaccines targeting respiratory viruses may reduce *S.pneumoniae*-associated diseases in Vietnam.

2487

Spotted Fever Group Rickettsiae in Ectoparasites from Northwestern Peru

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Rickettsial diseases have reemerged as a significant public health threat in South America, including one recently documented outbreak. *A. maculatum* and *I. boliviensis* were collected during the investigation of that outbreak and were shown to be infected by a novel species of spotted fever group rickettsiae, *Candidatus "*Rickettsia andeanae".
The purpose of this study was to determine the distribution and prevalence of this new SFG rickettsia in ectoparasites collected from domestic animals in northwestern Peru.
Sampling was conducted in three districts in the Department of Piura: Paimas, Suyo and Tambogrande. Ectoparasites including fleas, ticks and lice were removed with fine forceps from horses, cows, goats, pigs, dogs and guinea pigs. Specimens were separated by sex and life stage, identified, and then frozen at -80ºC. DNA was extracted from individual ectoparasites, pooled (3 to 6 specimens per pool) and tested for the presence of rickettsiae using a quantitative real-time PCR (qPCR) assay specific for the 17-kDa antigen gene found in all species in the genus *Rickettsia*. DNA from individual ticks were then tested with another qPCR assay specific for *ompB* of *Candidatus* "R. andeanae". We also sequenced part of *gltA* (citrate synthase gene) and *ompA* of the rickettsiae.
A total of 742 ectoparasites were collected including 727 ticks (*A. maculatum, An. nitens, B. micropilus, Rh. sanguineus* and *O. megnini*), 3 fleas (*Ct. canis*) and 12 pig lice (*Anoplura sp.*) from 145 domestic animals. We detected the presence of rickettsiae in 37 of the 742 samples.
Candidatus "R. andeanae" was found in 26 *A. maculatum* ticks and in 2 *Rh. sanguineus* ticks, and *Rickettsia parkeri* was detected in 7 *A. maculatum* ticks. *Rickettsia felis* was found in 2 of the 3 fleas. *Candidatus* "R. andeanae" was found distributed in all three districts of Ayabaca Province, and it was sympatric with *R. parkeri* and *R. felis* in Paimas. These findings expand the natural arthropod vector of *Candidatus* "R. andeanae" to include *Rh. sanguineus*, as well as expand the geographic range of *R. parkeri* to include Peru.

2488

Concurrent infections by dengue virus serotypes during an outbreak of dengue in northwestern in Peru, during 2008.

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The co-circulation of all dengue virus serotypes is a frecuent ocurrence in many countries of the world including Peru, however concurrent infection with more than one serotype of dengue viruses in the same patients is rarely documented. An outbreak of dengue fever/dengue hemorrhagic fever (DF/DHF) occurred in northwestern in Peru during 2008. DEN1, DEN3, DEN4 serotypes were found to be co-circulating in this outbreak with DENV3 being the predominant serotype. In addition in 6 of 27(22%) dengue virus positive samples, concurrent infection with DEN1 y DEN3 serotypes were identified. This is the first report in which concurrent infections with two different serotypes is being reported during an outbreak from Peru, where to exist the circulation of all dengue virus serotypes.

2489

Intestinal Parasites and Associated Risk Factors in Asendabo, Southwest Ethiopia

Intestinal parasitic infections have become a significant health concern within African Sub-Saharan populations. Morbidity and mortality due to parasitic infections include malabsorption, diarrhea, anemia as well as stunting of linear growth and cognitive development in children. Previous studies have been done detailing the prevalence of GI parasitic infections in other countries. No study has been conducted in regards to the prevalence in Southwest Ethiopia, specifically in Asendabo, Ethiopia.
Data was gathered between Sept 1st and December 30th 2008. During this time, approximately 827 people volunteered to submit stool samples to determine the prevalence of GI infections. In addition, the volunteers were asked to fill out a questionnaire to obtain possible risk factors associated with GI infections. Specific risk factors include personal hygiene, use of communal latrines, source of water and shoe wearing.
Based upon stool samples collected from volunteers, there were was 91.3% prevalence of intestinal parasites. Of the parasites discovered to cause infection, *Hookworm species*, *Ascaris lumbricoids* and *Trichiuris trichiura* were found to be significant in that order. Furthermore, it was discovered that the availability of latrines, source of drinking water, personal hygiene and shoe wearing had statistically significant correlation with GI infections.
Based upon our findings we conclude this study to be the foundation of future research involving parasitic infection within the Sub-Saharan population. Furthermore, we have the groundwork to develop interventional and preventative programs to decrease the prevalence of GI infections. Hence, decreasing the instances of morbidity and mortality due to parasitic infection.

2490

Knowledge, Attitudes and Practices on Malaria Control Interventions - insight from 18 Districts in Zambia

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Introduction: Malaria is a public health concern in Zambia. A package of interventions has been scaled up in Zambia and as a result the country is poised to make great strides in reducing the malaria burden. The interventions are both preventive and curative. Zambia has seen a dramatic decrease in the number of malaria cases, with 10% country estimate of parasitemia. A survey was conducted in 18 districts to determine knowledge, perceptions, attitudes and practices regarding the use of the malaria control interventions.
Method: A cross sectional survey was conducted, which employed both qualitative and quantitative methods of data collection.
Results: 1487 respondents were interviewed during the survey. 80% indicated fever as the main malaria symptom. The other symptoms included vomiting (57.1%), diarrhea (22.1%), pale eyes (13.2%) and refusal to eat/drink (27%). 78.6% of the respondents indicated that mosquitoes cause malaria. Other responses included eating sugar cane (6.4%), eating cold nshima (2.1%), eating dirty food (5.3%), drinking dirty water (15.1%), being soaked by rain water (10.1%), cold weather (17.7%) and witchcraft (1.1%). ITNs were found to be the most preferred malaria control intervention (73.7%). 13.7% of the respondents preferred IRS; however 11% preferred the use of all available interventions. The use of IRS was known to 52% of the respondents while 100% knew about ITNs. 90.2% of the respondents knew that pregnant women were vulnerable to malaria and 79.3% knew that pregnant women needed to take SP for IPT. 82.5% of the respondents went to health centers when they suspected having malaria while 13% opted to buy medicine and self treat.
Conclusion: Understanding the knowledge, attitudes and practices of the target populations is critical in assessing malaria control programs. The most preferred prevention intervention is ITNs; this could be due to the fact that the IRS program is currently in only 50% of the districts. There was an appreciable understanding of the various malaria control interventions. However some misconceptions still exist. Uptake of health services from the public health facilities was quite high. The National Malaria Control program should thus continue with its efforts in scaling up the interventions and should continue to educate communities on the importance of these interventions. Appropriate information is essential on acceptability and utilization of all malaria control efforts.

2515

Detection of ESBL in Urine Specimens from Hospitalized and non-Hospitalized Patients at Hamad Medical Corporation in Doha- Qatar

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Background
Extended -Spectrum Beta-Lactamases (ESBL) producing organisms are among the fastest growing problems in the area of infectious diseases. These Beta-Lactamases can be produced by a variety of *Enterobacteriaceae*; however, the most common ESBL-producing organisms are *Klebsiella pneumoniae*, other *Klebsiella* sp (i.e.,*K.oxytoca*), and *Escherichia coli*. With the ability to produce highly effective Beta-Lactamases, these organisms are resistance to all Beta-lactam antibiotics except cephamycins (cefoxitin, cefotetan) and carbapenems. In addition, ESBL-producing organisms are frequently resistance to many other classes of antibiotics, including aminoglycosides and fluoroquinolones. Hence, a more appropriate name would be "Multidrug Resistance Organisms".
Material & Methods
Urine specimens were inoculated on both CLED and McConkey agar medium by urine sample using a calibrated loop of the undiluted urine through different methods of collection where the majorities were MSU. Plates were incubated at 35 ± 2°C for 18-24 hours. Colonies of lactose fermenters were carefully selected and antibiotic disk diffusion test on Muller Hinton agar using three antibiotics Cefoxitin (FOX), Amikacin (AMC), and Cefotaxime (CTX) were made and the findings were confirmed by Phoenix automated microbiology machine.
Results & Discussion
Three hundred and seventy urine samples were collected that were enterobacteriaceae positive, where thirty eight specimens (9%) turned out to be ESBL positive. Where twenty eight (74%) *E.coli*, five (13%) *K.pneumoniae*, two (5%) *Citrobacter frendii*, two (5%) *Enterobacter cloaca*e, and one (3%) *Acinetobacter lwoffii*. The current data were comparable to those in Suadi Arabia and UK.
Conclusion
ESBL producing *E.coli* that causes UTI is much more common than the ESBL producing *K. pneumoniae* that causes UTI. The infections rate was more in adults with a slightly increased incidence in females. Qatari nationals had more prevalence rate of infection than non-Qatari and mostly were among non-hospitalized patients.

2516

The influence of helminths on immune responses to HIV

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In South Africa, co-infection with HIV and intestinal parasites is a major challenge in disadvantaged communities who live in densely populated under serviced urban informal settlements. This pilot cross sectional study evaluates the immunological effects of co-infection with *Ascaris lumbricoides* and *Trichuris trichuria* on the immune response to HIV.
The profile of lymphocyte phenotypes, viral loads, eosinophils, activation markers, expression of the nuclear proliferation antigen-Ki67, activation regulator antigen CTLA-4 were analysed using flow cytometry in HIV positive and negative subgroups with or without helminth infection. The type-1, type-2 and inflammatory cytokines were analysed using multiplex technology to determine the impact of helminths on the profile types expressed. These were correlated with immune responses to HIV. Non parametric statistics were used to describe differences in the variables between the subgroups.
The presence of helminth stool eggs and high Ascaris IgE (egg^+/IgE^{hi}) was associated with reduction in all lymphocyte populations; frequent eosinophilia; highly activated profile and antigen specific proliferative hyporesponsiveness; impaired type 1 cytokine responses in unstimulated and antigen stimulated cells; and increased TNFα levels. In HIV infected individuals, the egg^+/IgE^{hi} helminth infection status was associated with low $CD4^+$ counts and higher viral loads. A strong negative correlation was observed between viral loads, $CD4^+$ and $CD8^+$ cells in this subgroup.
Subgroups with high IgE (egg^+/IgE^{hi} and egg^-/IgE^{hi}) had elevated Th_2 markers and were associated with lower $CD4^+$ counts and higher viral loads in the HIV^+ group. The inverse correlation between viral load and $CD4^+$ counts found in all the HIV^+ participants was strongest in these two subgroups. The high IgE and HIV co-infeced subgroups presented a more activated profile compared to low IgE responders. Individuals with parasite eggs in stool and low Ascaris IgE (egg^+/IgE^{lo}) presented a modified Th_2 profile. This subgroup had high absolute numbers of all lymphocyte subsets in both HIV^- and HIV^+ groups with higher $CD4^+$ counts in the HIV^- and lower viral load in the HIV^+ groups as well as higher interferon gamma, lower IL-4 and higher IL-10. In conclusion, the results suggest that helminth infections may be associated with deleterious effects on the immune responses to HIV in certain groups of susceptible individuals.

2529

Arsenic Risk Mapping in Bangladesh

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Drinking water containing traces of arsenic poses a great threat to public health. Long term exposure to drinking water causes cancer (skin, bladder, lung, kidney and liver) and even death from gastric bleeding. In Bangladesh, dermal and internal effects have also been reported. Bangladesh Demographic and Health Survey (BDHS), 2004 tested drinking water in 10,465 household to examine the levels of arsenic in the drinking water. In Bangladesh, the drinking water standard is 50 ppb. The BDHS survey found that significant regional variations exist in distribution of unsafe levels (≥50 ppb) of arsenic in drinking water across the country. The percent of households with unsafe drinking water ranged from 2.3% in Rajshahi to 22% in Chittagong. It is widely believed that the arsenic levels in water vary over time and that treated or stored household drinking water may have different levels of arsenic compared to the source of water. Therefore, the geo-spatial distribution of arsenic risk may also differ based on whether it represents the source of water or the drinking water in the households. We conducted spatial comparison using the Kappa statistic to determine patterns of arsenic levels from the BDHS household testing of drinking water and compared it to the arsenic levels from shallow water sources of the U.S. Geological Survey (USGS) mapping. The paper will also present the findings of the re-aggregated DHS data based on the arsenic zones from USGS mapping and how it relates to other socio-demographic and health variables including information on the knowledge of arsenic exposure among the survey respondents.

2530

Impact of a comprehensive education campaign on community knowledge regarding Dengue Fever in a rural Guatemalan community.

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A comprehensive community-based Dengue education and prevention campaign was conducted from June ‘08 - February ‘09 in the Dengue endemic municipality of Ipala, Dept of Chiquimula, Guatemala (14°37’N 89°37’W). The purpose of the campaign was to educate the population on Dengue disease and engage the community in efforts to reduce vector populations. The campaign enlisted two full-time Dengue educators who worked with local officials on programs including educational outreach to community and local government agencies, medical service providers, educators and students. The Dengue educators also made face-to-face house calls to several hundred homes over the course of the program. Public education efforts were made through frequent informational messages

on local radio and television stations and with widely distributed printed materials (posters, flyers and community billboards). To measure the impact of the program on Dengue-related knowledge, adults from 200 homes (~ 10% of the homes in the target area) were randomly chosen before and after the campaign for personal interviews using a standard questionnaire. Pre- and post-campaign data illustrate that, while most people had already heard of Dengue, the campaign was effective in increasing the general knowledge of residents. Compared to <50% pre-campaign, 100% of respondents claimed to be able to recognize *Aedes* larvae and knew that *Aedes* were day-time feeders post-campaign. Prior to the campaign, respondents identified their primary sources of Dengue information as radio/television (56%) and ministry of health personnel (69%). After the campaign, radio/television was identified as the primary information source by 20% and ministry of health personnel by 27% of respondents. In contrast, Dengue educators were listed as the primary source of information by 53% of respondents post-campaign compared to only 1% pre-campaign. The results of this study indicate community-based education programs can be effective in increasing general knowledge in the community and suggest that, at least in this part of Latin America, dedicated Dengue educators are more effective than some traditional approaches to community education efforts.

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Isolation, Identification and Characterization of Influenza Virus strains circulating in children in the Greater Accra Region.

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INTRODUCTION
Influenza, an acute febrile self-limiting viral infection of the upper respiratory tract, is a leading cause of respiratory tract infections among children. In Ghana, though acute respiratory illnesses are second only to malaria for outpatient care, studies on causative agents have been limited. This study sought to characterize influenza virus strains circulating in children in selected hospitals in the Greater-Accra Region of Ghana.
METHODS AND SUBJECTS
Nasopharyngeal aspirates were collected from children, 5 years and below, presenting with symptoms of acute respiratory tract infections at hospitals in the Greater Accra Region between June 2008 and January 2009. Case definition for recruitment of study subjects after informed consent comprised body temperature >38°C, cough and coryza. Samples were inoculated onto Madin Darby Canine Kidney cell cultures. Haemagglutination inhibition assays with Influenza A and B reference antisera were used to identify viral isolates. RNA extracts of nasopharyngeal aspirates were subjected to real time Reverse Transcriptase Polymerase Chain Reaction to determine the presence of influenza genomes. Viral isolates were also subjected to sequencing analysis.
RESULTS
Of 62 children studied; 53.2% were males with mean age of 24 months and 46.8% were females with mean age of 28 months. Of the seven (11.3%) influenza viruses isolated, two (28.6%) were identified as subtype H1N1 similar to A/Brisbane/59/2007 (H1N1), one (14.3%) as an H3N2 subtype similar to A/Brisbane/10/2007 (H3N2) and the remaining four (57.1%) were Influenza B similar to B/Florida/4/2006 (Yamagata lineage).
CONCLUSION
The presence of these Influenza A and B viral strains in these Ghanaian children is in consonance with current global trends. However, phylogenetic analysis showed that Northern hemisphere vaccine strains are not well matched with vaccine strains isolated in Ghanaian children. Data obtained indicates that Influenza A and B viruses are contributing to acute respiratory tract infections among children in the Greater Accra Region.

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SHORT COURSE PAROMOMYCIN TREATMENT OF VISCERAL LEISHMANIASIS IN INDIA : 14 VERSUS 21 DAY TREATMENT

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Abstract
Background -Treatment of visceral leishmaniasis (VL) is far from satisfactory. There is an urgent need of a therapy which is efficacious, safe, affordable, and of short duration.
Method - A randomized, open label, study was conducted to assess the efficacy and safety of two regimens of paromomycin administered intramuscularly: Group A - 11 mg/kg/day for 14 days (n= 217) and Group B - 11 mg/kg/day for 21 days (n= 112) for the treatment of VL in India.
Results - Mild grade injection site pain was the commonest adverse event, there was no nephrotoxicity but four patients in Group A had to discontinue treatment due to grade 3 elevation of hepatic enzymes. Initial cure was observed in 91.2% and 96.4% patients in Group A and B, respectively. Definitive cure, at six month follow up, was seen in 82% and 92% patients by intention to treat analysis, and in 84.3% and 92.8% by per protocol analysis, in Group A and B, respectively.

Conclusion - Although the cure rate with the 14 days regimen was not optimal, the results of the initial cure were encouraging. Further studies combining a short course of paromomycin with another antileishmanial agent are warranted.

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Correlation of Alpha-Tubulin II and *Pf*g377 Ortholog Gene Expressions in *Plasmodium vivax* Gametocyte and Mosquito Infection

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An index of infectiousness based solely on an analysis of gametocyte counts from blood films is used as a malaria surveillance metric. However, the correlation between gametocyte density and mosquito infection is often ambiguous. Thus, the gametocyte count method is an unreliable tool for accurately predicting mosquito infection. In *Plasmodium falciparum*, alpha-tubulin II and *pfg377* are gametocyte-specific genes essential for microgametocyte formation and the emergence of macrogametes from erythrocytes during the gametogenesis process, respectively**.** An aim of this study was to determine whether the expression of stage-specific genes in malaria gametocytes would be useful for predicting infectiousness in the mosquito vector. The mRNA expression of alpha-tubulin II and *pfg377* ortholog in *Plasmodium vivax* was first analyzed by quantitative real-time PCR assay from 74 blood samples of vivax malaria patients in Tak province, Thailand. Laboratory-reared *Anopheles dirus* mosquitoes were fed on infected blood samples and infectiousness was determined by oocyst counts 7 d after mosquitoes were blood-fed. Results indicate that there was a correlation between mRNA expression of alpha-tubulin II, *Pfg377* ortholog genes and mosquito infection (R= 0.724, $P < 0.001$ and R= 0.471, $P< 0.001$ respectively) while gametocyte counts did not correlate with mosquito infection. In conclusion, the expressions of alpha-tubulin II and *pfg377* ortholog genes can be used as potential markers for accurately determining *P. vivax* gametocyte infectiousness when implementing malaria surveillance measures.

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Comparison of Costs Incurred in Dedicated and Diffused Vaccine Logistics Systems: *Analysis of Vaccine Logistics in Cabo Delgado and Niassa Provinces, Mozambique*

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Costs of last-mile vaccine logistics in the public sector of low resource countries are largely unknown. Cost data is difficult to access and studies at the rural level are virtually unheard of. VillageReach performed a cost analysis of vaccine distribution systems to rural health centers in order to compare the costs of a dedicated logistics system in Cabo Delgado province of Mozambique, to the existing diffuse system in neighboring Niassa province. The logistics system in Cabo Delgado, implemented by VillageReach, a US-based NGO, established an active dedicated vaccine distribution system with centralized staff and resources. This system was compared to the ad hoc mixed and unreliable system of collection and distribution activities that exist under government management in Niassa province. The comparison showed both an absolute cost savings under the dedicated system as well as savings on a cost per child basis as measured by children vaccinated with DPT-Hep B3 and all children under age five. The dedicated system was 23.2% less expensive overall, but when coupled with the significantly higher coverage rates experienced using the dedicated system, it was 44.4% less expensive on a cost per child vaccinated basis ($5.76 per child under the dedicated system vs. $10.36 under the diffused system). This paper provides an operational analysis of both systems, details the logistics platform and management information system implemented to yield the results, and offers recommendations for the design and implementation of dedicated logistics systems for other last-mile health systems globally.

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Interacting environmental and programmatic status determinants of coverage with larval stage mosquito surveillance in Dar es Salaam, Tanzania

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Introduction:
Controlling vector mosquitoes in their larval stages for malaria control is logistically complex. Here we assess the quality of a community-based larval control program by examining interacting environmental and programmatic determinants of coverage with larval surveillance.
Methods:
The Urban Malaria Control Program (UMCP) in Dar es Salaam, Tanzania delegates responsibility for routine mosquito control and surveillance to community members, known as Community Owned Resource Persons (CORPs). A trained mosquito biologist initially

conducted an independent cross-sectional quality control assessment of larval surveillance by 64 CORPs.

Results:
Only 7.8% of the 2965 wet habitats found by the investigator were occupied by any aquatic stages of *Anopheles*. CORPs had detected almost 66.2% of wet habitats. The detection sensitivity for occupation of habitats by aquatic mosquito stages by CORPs was low, ranging from 15% to 30% but was particularly poor for late-stage Anopheles (2.7%; 3/111). The distribution of habitat types varied differently between fenced plots and unfenced plots (χ^2 = 50.037, df =10, P<0.001) and the former had drastically reduced detection sensitivity for early-stage *Anopheles* (OR [95% CI] = 0.07 [0.010, 0.728] and all stages of Culicines (P<0.05). One fifth (20.5%) of aquatic habitats occurred in plots with which the CORPs were unfamiliar and this reduced their detection probability (OR [95%CI] = 0.16 [0.13, 0.21], P<0.001.
Discussion: Although detection levels of habitats by CORPs have improved through vertical management, coverage remains incomplete. Moreover, detection of mosquito larvae by CORPs was very poor. Improved supervisory and quality control systems are urgently required to maximize operational impact.

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An exposure-free tool for monitoring adult malaria mosquito populations

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Background.
In the drive to eliminate malaria, mosquito sampling measures are crucial to monitor human exposure to infections and the effect of vector control interventions. Recently, a new device for sampling *Anopheles* called the Ifakara tent trap-design B (ITT-B) has been developed and evaluated in Tanzania as an alternative to human landing catch (HLC). The relative sensitivity of ITT-B increased as vector densities decreased and exceeded that of HLC at the lowest densities. ITT-B also correlated better to HLC than any other tested method, and is remarkably cost-effective under programmatic settings with minimal supervision. However, ITT-B failed to reduce the proportions of blood-fed mosquito caught relative that from HLC, indicating operators were exposed to mosquito bites and malaria transmission.
Methods
Ifakara C trap (ITT-C) was derived from the original ITT-B by modifying the trap chamber so that mosquitoes can be removed without opening it. The ITT-C was evaluated against the ITT-B in both semi-field and field conditions using cross-over experimental design in rural Tanzania
Results
The crude mean sensitivity of the ITT-C for *An. gambiae sensu lato, An. funestus, Culex sp, and mansonia sp* relative to ITT-B =2.27, 20.00, 2.01 and 1.75 respectively. The ITT-C caught far less blood-fed *An. gambiae* s.l. (Odds ratio [95% Confidence Interval] =0.27[0.12, 0.60], P = 0.001 relative to ITT-B) in the field and none (0.0% (0/190) compared with 3.5%, (6/171) for ITT-B) in semi-field.
Conclusion and Recommendations
The Ifakara C is a genuinely exposure-free method with great potential for monitoring under programmatic conditions. Validation by comparison with HLC in terms of predicting human infection rates may be necessary to confirm that this design produces results which are most epidemiologically meaningful.

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Spatial pattern of Dengue Occurrences in Coastal area of Thailand

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Background Without an available vaccine, lessening dengue vector is an only measure of dengue control which is most effective through community-based bottom-up approach. It remains a challenging task particularly in resource-constraint area because this measure needs political power and high budget. Applying an analysis of the spatial patterns of dengue cases can identify high risk area, and allows public health authority to efficiently allocate resources.
Methods Five-years report cases of dengue in Prachuap Khiri Khan, Thailand, were obtained from provincial health office. An empirical Bayes estimation was employed to estimate village-specific incidence rate in the study region.
Result Half of villages in the study area reported at least one dengue cases. Spatial analysis presented that the south-eastern part of Prachuap Khriri Khan had a higher density of dengue cases than other parts.
Conclusion Our results showed that, in village level, higher density of dengue tended to occurred in new city areas. Use of geographic tools and spatial analysis can provide information for evaluating the effectiveness of dengue prevention and control programs in a local scale.

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Linking the ecology and genomics of malaria using ontology and generic schemas

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To build solid programs which will be able to eliminate malaria, scientists need to be able to analyze huge quantities of ecological and genomic data in order to understand the complex relationships between the malaria parasite, mosquitoes and humans. Recent advances have been made regarding the archiving of genomic data; but informatics tools for ecological data are still in their infancy due to the huge quantity of data formats and the heterogenous nature of ecological data. As such, we developed a formal ontology for the classification of ecological and genomic data about malaria vectors. Our aim was to develop a framework that would enable the robust description, classification and synthesis of ecological and genomic data.
The most important stage is during data collection and thus we have designed a series of generic schemas to assist scientists to record high quality experimental data. The schemas have been designed as a multipurpose template that provide structure to data collection while at the same time flexibility for the user to select which attributes will be recorded during each specific experiment. The schemas can be used when collecting eggs, larvae, pupae or adult mosquitoes from the field or when conducting laboratory and semi-field based experiments. They have been designed for both paper-based and electronic data collection. The electronic data collection methods (PDA and mobile phones) have proved extremely successful and have vastly improved the quality and reporting time of data. For example, the mobile-phone based system was recently implemented in Dar es Salaam, Tanzania, for use by the surveillance teams employed within the Urban Malaria Control Program. The adult and larval density data is entered into specially programmed phones, after which a text message is sent to the server, the data is instantaneously uploaded into the database and weekly reports are automatically created. This system has vastly reduced the time delay from data collection to reporting allowing more rapid responses of program managers on a real-time basis.

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Double-blind, randomised, controlled trial on iron supplementation in anaemic HIV infected Malawian children: Is it safe and beneficial?

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Introduction:
Iron supplementation is a relatively cost-effective strategy for preventing and controlling anaemia. Conversely there is evidence that iron supplementation may be associated with increased morbidity in iron depleted children. HIV infected anaemic children may benefit more from iron supplementation since they are more likely to be micronutrient deficient. However, iron supplementation may carry the risk of increasing the susceptibility to infection in children with a compromised immune system.
Methods:
We are carrying out a double blind randomized controlled trial of multi-vitamins with iron supplementation versus multi-vitamins alone in HIV infected children. Children (6- 59 months of age) are being recruited from HIV clinics & paediatric wards in two hospitals in southern Malawi and randomized to receive recommended therapeutic doses of oral iron, 3mg/kg/day as a concentrate with multi-vitamins or multi-vitamins alone for 3 months. They are being actively and passively followed up for a period of 6 months. The main safety outcomes are the incidence of sick visits, hospitalizations and deaths. A total of 1340 (670 children in each intervention group) will be recruited.
Results and Conclusion:
The trial is on-going, recruitment of trial participants started in January 2009. It is hoped that the results of this trial will help direct policy in the area of iron supplementation for children in areas endemic for iron deficiency, malaria & HIV.

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Protein deficiency and nematode infection during pregnancy and lactation modify maternal body composition and impair linear growth in the murine neonate

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This study investigated whether diet or trickle nematode infection during pregnancy and lactation influenced maternal body and bone composition, and whether maternal stress would impair neonatal axial skeletal growth in CD1 mice. Using a 2 X 2 design, pregnant mice fed either protein sufficient (PS, 24%) or deficient (PD, 6%) diets were infected four times with 0 (sham) or 100 (infected) *Heligmosomoides bakeri* larvae. We measured whole body and lean mass, bone area (BA), bone mineral content (BMC) and bone

mineral density (BMD) of dams, serum markers of bone remodelling in dams and pups, pro-inflammatory cytokines in maternal serum and pup serum and milk, and corticosterone in maternal serum and urine, and pup serum and milk. Linear growth and serum IGF-1 were also measured in pups. PD reduced whole body, lean and fat mass, BA and BMC, perhaps as a result of the corticosterone-induced elevation of leptin in maternal serum. Contrary to expectation, serum RANKL (marker of bone resorption) was lower in PD dams. The infection-induced reduction in maternal BMD and osteocalcin (marker of bone formation) was consistent with elevated pro-inflammatory cytokines IL-1β and IL-6 in response to *H. bakeri* infection. In pups, maternal infection reduced both IGF-1 and leptin in pup serum. Furthermore, maternal PD reduced pup serum IGF-1. These results are consistent with the reduced crown-rump length of pups in response to maternal infection and PD.

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***PLASMODIUM VIVAX* - APICOPLAST GENOME**

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***PLASMODIUM VIVAX* - APICOPLAST GENOME**
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Plasmodium vivax is responsible for causing greater than 50% of human malaria cases in Southeastern Asia and the Indian subcontinent. The rising severity of the disease and the resistance shown by the parasite towards usual therapeutic regimen has put forth a demand for a novel drug target to combat this disease. Apicoplast, an organelle of prokaryotic origin, and its circular genome are being looked upon as a potential drug target. The Apicoplast genome is known to carry various genes of functional importance. Except for a few reports, this genome has not been detailed from *P. vivax*. Our group for the first time has reported any complete gene (*tufA*) from this genome of *P. vivax* (Saxena et al., 2007). In the present study we have characterized other major genes of this genome, including *ssu* and *lsu* ribosomal RNA and *tRNA* genes, *sufB*, *clpC*, genes, *RNA Polymerase B*, *C* and *D* subunit genes and various ribosomal protein genes.
The Apicoplast genes were sequenced and analyzed from *P. vivax* field samples. A comparative analysis of *P. vivax* Apicoplast genes with alleles from other *Plasmodium* species (especially *P. falciparum*) showed about 8 - 13% differences at both nucleotide and amino acid level. To colocalize the Apicoplast within *P. vivax*, peptides were designed from the *P. vivax* Apicoplast *tufA* gene and antibodies were raised in swiss albino mice. Apicoplast was colocalized using antibodies raised against Ef - TuA peptides in *P. vivax* infected blood smear slides obtained from the field.
Reference: Saxena V, Garg S, Ranjan S, Kochar D, Ranjan A, Das A. 2007. Analysis of elongation factor Tu (tuf A) of apicoplast from Indian *Plasmodium vivax* isolates. Infection, Genetics and Evolution 7: 618-626.

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Recombinant anti-microbial peptide (AMP) expression and toxicity to *Trypanosoma cruzi* from transformed *Rhodococcus Rhodnii* for application to the paratransgenic model of Chagas disease control

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Insect-transmitted infectious diseases remain a leading cause of morbidity and mortality despite great advances in public health world-wide. Paratransgenic methods of disease eradication target specific infectious agents within insect vectors using genetically altered symbiotic bacteria expressing anti-pathogen molecules. Chagas disease, caused by the protozoan parasite *Trypanosoma Cruzi*, is carried and delivered by triatomine bugs including *Rhodnius prolixus* that harbor infective *T. cruzi* in their hindgut lumen in close proximity to the symbiont *Rhodococcus rhodnii*. Previous studies showed that Transformed *R. rhodnii* expressing the antimicrobial peptide, cecropin A, within the body of *R. prolixus* could clear *T. cruzi* infection in 65% of the paratransgenic insects examined, while the remainder had substantially reduced titers.
Cecropin A is an anti-microbial peptide (AMP), a growing class of amphipathic, basic molecules that include apidaecin, magainin II, melittin, and moricin. These AMPs have been found to be paracidal to *T. cruzi* in our lab, exhibiting additive and synergistic toxicity profiles when applied in pair-wise combinations. The DNA sequences for these AMPs were cloned into Rhodococcus/*E. coli* expression shuttle vectors and transformed into electro-competent *R. rhodnii.* AMP expression was confirmed by western blot and ELISA assays of culture supernatants and cell extracts. Treatment of *T. cruzi* cultures with the cell extracts and supernatants from recombinant *R. rhodnii* show anti-Trypanosomal activity by light scatter analysis and fluorescent staining of treated parasites with the Calcein-AM live cell indicator.

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A transgenic mosquito delivering a Leishmania vaccine antigen via blood meal

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A concept of haematophagous insects engineered genetically to deliver protective vaccines via blood meal has been proposed. This concept has been known as a "Flying vaccinator" or "Flying syringe". However, in haematophagous insects including the mosquito, a salivary gland-specific expression system essential for this concept has not been developed. Recently, we have identified a strong salivary gland-specific promoter (the aapp promoter) from Anopheles stephensi mosquito, and established a robust salivary gland-specific expression system using mosquito transgenesis. In the present study, we generated a transgenic An. stephensi that expresses Leishmania vaccine antigen SP15 under the control of the aapp promoter. SP15 is known as a sand fly saliva protein and has shown promise as a protective molecule against leishmaniasis. The gene encoding SP15 fused to monomeric DsRed (mDsRed) was expressed in secretory cells of the female salivary glands, and secreted into the secretory cavities and duct. During salivation, mDsRed-SP15 fusion protein with red fluorescence was released from the proboscis. Most importantly, mice repeatedly bitten by transgenic mosquitoes raised anti-SP15 antibodies, indicating that mDsRed-SP15 fusion protein was injected as a component of saliva with its immunogenicity via blood meal. Thus, we achieved the original concept of "Flying vaccinator" as a model of Leishmania vaccine. With medical safety concerns against the current vaccination programs, however, the "Flying vaccinator" remains an unacceptable approach to the delivery of vaccines. Here we should clearly state that this is a model study of the expression of foreign genes in saliva and provides a useful tool for elucidation of saliva-malaria sporozoite interaction.

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Evidence of *Orthopoxvirus* Exposure in a Rural Amazonian Population, Brazil.

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Vaccinia virus (VACV) strains from the genus *Orthopoxvirus* (OPV) of the family *Poxviridae* have been frequently isolated, primarily in Southeastern Brazil, and associated with outbreaks of exanthematic disease affecting cows and humans. Worldwide, the seroprevalence of OPV has been accessed either as a marker for immunity elicited by vaccination or as an indicator of wild OPV exposure. In Brazil, it has been accessed only in populations with recorded outbreaks and the prevalence in populations otherwise naïve for OPV is far unexplored. Ramal do Granada is located in the eastern corner of Acre state, Northern Brazil, inside the largest governmental-funded rural settlement in the state. The main goal of this work was to evaluate the seroprevalence of orthopoxviruses in Ramal do Granada, where no orthopoxvirus outbreaks have yet been reported. To this end, an indirect ELISA IgG using VACV-WR as antigen was applied. The overall seroprevalence was 27.89% and higher in male subjects (OR: 2.0469, P= 0.0091) and older patients (P= 0.0236), although these two variables were not independent significant by multivariate analysis. Once the WHO smallpox vaccination campaign in Brazil ceased in 1976, we defined the cut-off of 27 years-old to segregate between those vaccinated and non-vaccinated individuals. Surprisingly, after analysis of the non-vaccinated population, a seroprevalence of 23.38% was found, and this was not correlated with sex, gender or the individuals' occupation. We speculate that activities promoting persons direct contact with natural areas, as timber cutting, rubber extraction or hunting could play a role in the infection's acquisition. These results strongly suggest that wild orthopoxviruses circulate in this population, likely as a non-occupational zoonosis. To our knowledge, this is the first finding of seropositive orthopoxviruses in a population without any previously reported outbreaks. This study may help to better understand and prevent the emergence of these pathogens that have an impact on human and animal health.

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Marked genetic diversity and differentiation among Glossina pallidipes (Diptera: Glossinidae) populations in Kenya

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We estimated genetic diversity at eight microsatellite loci among 539 Glossina pallidipes from nine populations in Kenya. Two of the populations were in western Kenya (Lambwe valley and Busia), two in southwest Rift valley (Narok and Nguruman), one in eastern Kenya (Kathekani), and four in coastal Kenya (Tsavo, Shimba Hills, Kwale, and Dakabuku).
No genotypic linkage disequilibrium was detected between any loci indicative of demographic equilibrium and selective neutrality. All loci were polymorphic and yielded between 12 and 41 alleles. In total, 198 alleles were detected with the mean number of alleles per locus being 24.8 ± 8.6. G. pallidipes diversities in Lambwe, Busia, and Nguruman Kenya were generally 1.4x less than the diversities of coastal populations as indicated by Nei's unbiased heterozygosity and the mean number of alleles. Such low diversities

can be attributed to earlier tsetse control efforts that caused reductions in effective (breeding) population sizes.
Genetic differentiation was high among all populations as measured by FST, the probability that two randomly chosen alleles are autozygous (overall FST = 0.10). Pair-wise comparisons of genetic distances between populations showed that G. pallidipes from Busia and Nguruman were the most genetically distant (FST ~ 0.33) whereas the least divergent populations were Kwale and Tsavo (FST ~ 0.01). These results indicate very limited gene flow between G. pallidipes in Busia and Nguruman (Nm~0.5), and regular exchange of individuals between Kwale and Tsavo (Nm~25.5). Tsetse habitats are highly discontinuous in mountainous Kenya but thorough mapping of suitable habitats is necessary before efficacious, long term control procedures should be applied. The implications of gene flow data for long term tsetse control are discussed.

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Effects of larval nutritional stress on vector immune traits in the yellow fever mosquito Aedes aegypti.

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Mosquitoes are vectors for many disease-causing pathogens. Larval stressors, such as malnourishment, can affect adult size that may then affect adult survivability and reproductive success. Our lab is investigating how larval nutritional stress on Aedes aegypti, an important vector of dengue virus, influences adult immune traits that can affect a female mosquito probability of infection. Little is known about anti-viral defenses in insects. We report on the effect of larval nutritional stress on immuno-responsive (IR) gene expression, including genes encoding for antimicrobial cecropins, defensins as well as genes encoding for components of the Toll and RNAi pathways. To examine transcript expression in larvae in response to nutritional status but not age, fourth instars were set up in specific feeding period regimen and larval carcasses were collected after 36 h. To examine transcript expression in females in response to nutritional status, females fed either high or low amounts of food as larvae were examined. Preliminary experiments based on RT-PCR and quantitative PCR indicate that nutritional deprivation as larvae results in reduced expression of the Toll pathway activation marker SpZ51 as well as a number of genes encoding for antimicrobial peptides. It is widely accepted that infection and dissemination barriers in the midgut largely determine mosquito competence for pathogens. The ultrastructure of the midgut lining seems to vary with body size. We are using transmission electron microscopy to examine the architecture and thickness of midgut epithelial lining in response to larval nutritional stress. In mosquitoes, synthesis of melanin is a general immune response that is used to encapsulate acquired pathogens. Phenoloxidase (PO) activity plays a key enzymatic role in the formation of melanin. Hemocyte population also plays an essential role in the mosquito immune system required for phagocytosis and encapsulation of foreign targets. We are beginning to investigate how these immune functions in Ae. aegypti are altered by poor larval nourishment.

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Doxycycline is effective in clearing microfilariae in the skin of onchocerciasis patients in whom repeated ivermectin treatment has failed to clear

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Introduction
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 (Osei-Atweneboana, 2007). These indications are consistent with the emergence of drug resistance or sub-optimal response to IVM. To assess the effect of doxycycline on onchocerciasis patients in whom repeated IVM treatment has 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 or matching placebo for 6 weeks. Three and 12 months after doxycycline treatment, all patients took part in ongoing IVM mass treatment. Patients were snipped before and 12 and 20 months after treatment to assess the levels of Mf that IVM could not clear.
Results
Before treatment, of the 73 patients allocated for doxycycline, 48 (66%) had persistent Mf in the skin and 25 (34%) had only nodules but no skin Mf, and of the 76 patients allocated for placebo, 48 (63%) had persistent Mf in the skin and 28 (37%) had only nodules (P=0.7408). However, at 12 months after treatment, out of the 72 doxycycline-treated patients snipped, only 7 (9.7%) had few Mf in the skin and 65 (90.3%) had no Mf at all. Of the 71 placebo patients snipped at the same time point, 41 (57.7%) had Mf in the skin and 30 (42.3%) had no Mf (P<0.0001). At 20 months post therapy, only 2 (2.9%) out of the 69 doxycycline-treated patients snipped had very low skin Mf and 67 (97.1%) had no Mf. In contrast, of the 71 placebo-treated patients, 49 (69%) still had Mf and 22 (31%) had no Mf, and there was a difference between the 2 groups (P<0.0001).
Conclusion
Doxycycline is effective in clearing Mf in the skin of onchocerciasis patients in whom repeated IVM treatment has failed to mediate Mf clearance, thus strategies may be developed including doxycycline to control the re-emergence of onchocerciasis in areas where infections persist despite the use of IVM.

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Willingness to pay for malaria risk reduction in four districts in Zambia.

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Willingness to pay for malaria risk reduction in four districts in Zambia.
Introduction: Malaria is a major public health problem and a leading cause of illness and deaths in Zambia. About 19% of mortality in under-five children and 5% maternal mortality is attributed to malaria. The malaria control programme in Zambia has scaled up both prevention and curative interventions. Progress indicators are improving; however the impact remains lower than expected. There is a need to understand the value that the targeted population attaches to risk reduction in malaria control. The study will also contribute to methodological debate on willingness-to-pay in health care in low income countries.
Methods: This was a cross sectional, contingent valuation to determine the mean willingness to pay for malaria risk reduction using household interviews. The study sites were four districts from four of the nine provinces of Zambia. The dichotomous choice questioning format using a double bound approach was used to elucidate the willingness to pay for any of the three risk reduction scenarios.Eight bid amounts were randomly selected for each risk scenario to ensure that the respondents were randomly distributed to any of the bid amounts. Regression analysis was applied to determine variables which impact on willingness to pay for reductions in malaria risk.
Results: Of all the people interviewed**,** 61% were female, 15% formally employed and 48% had some secondary education. Overall, 50%, 66% and 73% of the respondents were willing to pay for one, five and ten years of malaria elimination at first bid. These proportions improved significantly at second bid. The mean willingness to pay (USD 24-USD30) was higher than the mean household expenditure for malaria. The internal validity of the findings will be checked by regressing WTP on socioeconomic variables.
Conclusion: Households seem to value malaria risk reduction in children aged five years and below. More respondents were willing to pay for 10 years of risk reduction than one year. However, the mean WTP was not sensitive to the duration of the risk reduction.
Acknowledgement:s
Sincere thanks to Dr E. Chizema and Mr. B. Hamainza of the National Malaria Control Centre for their input and support. Funding for the study was from the MACEPA support to capacity building in Zambia. Appreciation goes to the DHMTs and research assistants who made this study possible.

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Evaluation of Functional Capacity Among Patients With Pulmonary Tuberculosis Using Six Minute Walk Test.

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PULMONARY SYMPTOMS AND FUNCTION OF ROAD TRAFFIC WORKERS IN NIGERIA
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Introduction
Lagos is a highly polluted metropolis and the industrial capital of Nigeria with a projected growth to 18 million persons by 2010. This growth is associated with an exponential rise in dust and particulate matter pollution, industrial flaring and vehicular emissions. Traffic workers who spend most of their working hours outdoor are particularly at health risk.
The study was aimed at evaluating the pulmonary function among traffic workers.
Method
Fifty one (51) traffic workers and fourteen (14) controls were randomly selected. The controls were age and sex matched students of University of Lagos as they reside in low pollution environment.
A modified MRC was administered to elicit the presence of respiratory symptoms and to obtain socio-demographic data. They all had pulmonary function testing and Carbon-Monoxide (CO) breath test using a CO breath analyzer according to standard guidelines.
Result:
Mean age ± SD 35±8years, BMI 25+4kg/m^2, FEV1 2.92 ± 0.97(L), FVC 3.66 ± 2.26(L), FEV1/FVC 103, CO Breath test 1.18±0.43ppm. There was a significant level of respiratory symptoms among the traffic workers compared with controls: Cough 53% Odd Ratio (OR) 0.84, Sputum 12% OR 0.81, Wheeze 8% OR 1.11, Chest tightness 10% OR 1.31, Shortness of Breath, 14% OR 0.58, Rhinitis 51% OR 0.58, Irritation of Eyes 49% OR 0.72. There was no significant difference in the lung function parameter but the CO breath test showed a significant high level among the traffic workers.
Conclusion.

This pilot study showed a high level of respiratory and non-respiratory morbidity among traffic workers in Lagos, Nigeria. There is the need for a more comprehensive study and adequate measures to reduce pollution and protect traffic workers.

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Compliance to antimalarial prophylaxis in Slovak humanitarian and medical personnel in Sub-Saharan Africa

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Compliance to antimalarial prophylaxis is a key issue in prevention of malaria in travellers. (1) We assessed compliance and efficacy of antimalarial drugs in 80 travellers and humanitarian aid workers, 24 were covered with prophylaxis - those who travelled to Uganda, Burundi, Sudan (1999-2009). After 2000, we have not recommended prophylaxis in Nairobi and Eldoret due to higher altitude (1900 m.a.s.l) and no cases of malaria among our staff within last years.
From 80 travellers, 8 developed malaria during stay (10%) none got malaria after travel. In 2 cases chloroquine, 1 doxycycline and 1 case pyrimetamin/sulfamethoxazol (1999-2000) have been used for 3-6 months 1 x weekly in Nairobi. In 20 other cases, mefloquine 1 x weekly has been used, 1 week before during and 1 month after the travel (Sudan, Ethiopia, Uganda, Burundi). From 20 courses, only 9 (45%) has been successfully completed, 11 were discontinued other 1-8 weeks because of adverse reactions - insomnia (3), depression (6), hallucinations (1), vomiting (1). Among 9 cases who completed prophylaxis, 4 developed malaria despite regularly taken prophylaxis with mefloquine (44.4%). Among 11 who did not completed prophylaxis (with mefloquine 1-8 weeks), 4 travellers developed symptomatic malaria (36.1%). They were no significant difference among those, who completed full course of prophylaxis and those who did not, with adverse reactions 44.4% vs. 36.1%, (NS, X^2 test). None of 8 cases of malaria were severe.
In conclusions our limited experience during 10 years of sending humanitarian medical personnel to Sub-Saharan Africa, shows, that work for 3-6 months in altitude more than 1800 m.a.s.l. does not represent a serious risk of malaria despite of reports of highlands malaria and prophylaxis with mefloquine did not influence the frequency of malaria cases. All but 1 case of symptomatic malaria occurred in Sudan, Uganda or Burundi. None of 8 cases in 80 travellers had severe clinical course of malaria and responded well to 3 days of oral arthemeter/lumefantrin therapy.

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Antibody Levels to Multiple Plasmodium falciparum Antigens in Adults from Urban and Rural Communities in Ghana by Multiplex Assay

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The importance of antibody in malaria immunity is well documented. Traditionally, ELISA is used to quantify antigen specific antibody levels. This assay may not be suitable when multiple antigen specific antibody assessments are required in a multi-antigen vaccine trial. Furthermore, sample volume may be limiting in multi antigen specific antibody measurements by ELISA in children who will be targeted for malaria vaccine. In this study, we used multiplex assay to assess antibody levels to 10 malaria vaccine candidate antigens (GLURP RO, GLURP R2, AMA-1 FVO, AMA-1 LR32, AMA-1 3D7, MSP-1 3D7, MSP-1 FVO, MSP_3 FVO, LSA FMPOII, and EBA-175 region II) in 18 and 20 adults (age 25 - 40 years) from urban and rural communities of Ghana, respectively. The samples were obtained during the low malaria transmission season. Optimal concentration of each antigen was coupled to microspheres with different light spectra, incubated with optimally diluted plasma and antibody levels measured as mean fluorescent intensities using Luminex^{100} (BIO-RAD, USA). AMA-1 LR32 and AMA-1 FVO showed the highest antibody level (range 4557.1 to 3778.6) followed by MSP-1 3D7, MSP-1 FVO, MSP_3 FVO, EBA-175, LSA FMPOII and AMA-1 3D7 (range 2876.1 to 993.1) while GLURP RO and GLURP R2 showed the lowest levels (range 830.4 to 491.4). Although, the levels of antibody to each of the antigens were similar between the two communities, a trend of higher levels of antibody response to EBA-175, AMA-1 LR32, LSA FMPOII, GLURP RO and GLURP R2 was found in rural dwellers while higher antibody levels were observed among urban dwellers for MSP-1 3D7, MSP-1 FVO, MSP_3 FVO, AMA-1 FVO and AMA-1 3D7. The similar levels of antigen specific antibody found in the two populations may be due to the decay of these antibodies in the rural dwellers to the level of urban dwellers during the low transmission period. Interestingly, positive correlations were found between antibody to AMA-1 variants (FVO and LR32) in the rural ($r=0.908$, $p=0.000$) and in the urban ($r=0.938$, $p=0.000$) populations. The result suggests that the AMA-1 variants (FVO and LR32) may have cross-reactive epitopes and/or parasite strains expressing such AMA-1 variants are in circulation in both sites. The study has shown that multiplex assay is suitable for use in preclinical malaria immunological studies and should be validated for use in antibody measurement during malaria vaccine trials.

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Antibody Levels to Multiple Malaria Vaccine Candidate Antigens in Relation to Clinical Malaria Episodes in Children Less Than Five Years in the Kasena-Nankana District of Northern Ghana

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Malaria is a devastating tropical disease causing high morbidity and mortality mainly among children under five years and pregnant women. The development of both parasite and vector resistance to anti-malarials and insecticides, respectively, has led to increased efforts in the search for novel control tools against malaria such as vaccines. IgG from African adults have been demonstrated to be effective in treating children suffering from clinical malaria by reducing parasiteamia and ameliorating fever. The acquisistion of anti-malaria antibody is both age and exposure dependent but the antigenic targets of these protective antibodies have not been conclusively identified and immunity to clinical malaria is thought be due to premunition where protective immunity is sustained in the presence of parasitaemia below clinical thresholds.
In this study, a cohort of children under five years were passively followed over a full malaria transmission season during which clinical, heamatological and parasitological data were collected bimonthly. Fingerprick blood was collected at the beginning of the study and bimonthly and the baseline blood sample was used to measure antibody levels to 10 malaria antigens (GLURP R0, GLURP R2, MSP3 FVO, AMA1 FVO, AMA1 LR32, AMA1 3D7, MSP1 3D7, MSP1 FVO, FMPOII LSA and EBA175RII) using the multiplex assay, a suspension array technique. The epidemiological data indicated a contant prevalence of about 80% asymptomatic parasiteamia throughout the period of study, with the exception of the end of the dry season when it was 60%. Multiple logistic regression analysis was used to determine the baseline characteristics most predictive of clinical episodes over the year. Upon correction for the confounding effects of age and baseline parasitaemia and/or geometric mean prasiteamia over the transmission period, only GLURP R2 and MSP1-3D7 were independently associated with protection against clinical malaria when the case definition included parasite density >2500 (p=0.036, p=0.029, respectively) or >5000 (p=0.04 for both antigens) parasites/ul blood. Parasite growth inhibition assays would be required to confirm if these associations reflect functional roles of antibodies to GLURP R2 and MSP1-3D7. Hierarchical statistical methods will be used to establish the relationship between multiple logitudinal measures of the antibody levels, parasitaemia, and clinical malaria.

2873

Assessment of Antibody Levels to Multiple Malaria Vaccine Candidate Antigens in Urban and Rural Areas of Ghana using a Standardized ELISA

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Malaria vaccines are being developed as an additional tool to control the disease and there is the need to assess the immunogenicity of the vaccine candidate antigens using standardized and validated assays, to enable effective comparison of data from trials with different vaccine antigens and at different sites. In this study, a standardized ELISA developed for use in assessing immunogenicity was used to quantify IgG responses to 7 malaria antigens (EBA 175 II, AMA1-3D7, AMA1-FVO, $MSP1_{42}$-3D7, $MSP1_{42}$-FVO, MSP2-257, and MSP2-259), using samples from volunteers aged 25-40 years in urban (Accra) and rural (Asutuare) areas in Ghana during low malaria transmission season. ELISA was performed for EBA 175II for at least 3 repeats by an experienced and 2 trainee operators. The data showed strong positive correlation ($0.985<\mathbf{r}<0.994$, $p<0.0001$) among the three operators. Pair wise analysis of the data from the trainees showed no difference between them (P=0.122). Average inter-person variations between the three repeats for each of the two trainees were 11.1%, and 14.6%, and between the 2 trainees was 13.9%. The variation between the trainees and the experienced operator was 9.4%. There were no differences in IgG responses to EBA175II in the two areas. The 6 other antigens showed similar IgG responses between the two sites except MSP2-257 which was higher in the volunteers from Asutuare (1165.04 ± 499.48) compared to the responses in those from Accra (964.85±510.54; $P<0.05$). For both urban (Accra) and rural (Asutsuare), respectively, there was correlation between the levels of antibodies to AMA1 ($r=0.967$ and 0.735, $p<0.001$) and that to MSP1 ($r=0.978$ and 0.819, $p<0.001$). Similarly, antibody levels to the variants of MSP2 (257 and 259) also correlated with each other ($r=0.835$, and 0.680, $p<0.001$) for Accra and Asutsuare, respectively, suggesting the circulation of both 3D7 and FVO variants of AMA1 and MSP1 in the two populations studied. This standardized assay is highly reproducible, requires small sample volume and will be used in the upcoming EBA-175 region 2 malaria vaccine trial where immunogenicity will be assessed by ELISA. It can thus be used to support vaccine trials.

2875

Highlands malaria in Eldoret, Kenya in altitude 2250 meters above sea level

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Highlands malaria, defined as malaria acquired in altitude more than 1500 meters above sea level. Baliraine study report 52.4% of malaria parasitemia in children living in 1430 meters above sea level and 23.3% in those living in 1580 meters above sea level. Similar experience has been described from Afghanistan in Kabul Province (1800 meters above sea level) in 2009 by Carmoi study. Our study group reported positive blood smear for *Plasmodium falciparum* in about 3000 adults and children among 12360 patients with clinical malaria from Eldoret, St. Lesley Tropical Clinic in Kenya, 2050 - 2250 meters above sea level. Majority (88%) of patients have not been travelling outside of Eldoret (2000 - 2250 meters above sea level).
The reason for increasing burden of malaria above sea level of 1500 meters is (i) global warming (ii) increased travel in some places in Sub-Saharan Africa (Ethiopia, Somalia, and Sudan) or displacement (iii) increased humidity and rain fall in mountains. Therefore, malaria has to be considered as cause of fever also in individuals living in altitude of 2000 meters above sea level, and even higher.

2876

Search for Secreted Immunomodulatory Proteins from Strongyloides

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The vital role of the balance between host immune and parasite evasion mechanisms can be strikingly demonstrated in Strongyloides. infection: after decades of inapparent chronic infection maintained by low level autoinfection of immunocompetent individuals treatment by immunosupressive drugs or HTLV co-infection can disturb this balance and lead to disseminated strongyloidiasis with fatal outcome in most cases. Excretory/secretory (E/S) products allow the nematode parasite to skew the immune mechanisms and thereby allow its survival and propagation while multiple innate and adaptive immune responses control the parasite. Therefore we performed mass spectrometry to identify excretory/secretory products of S. ratti. Amongst others we detected homologs of the heat shock proteins HSP10 and HSP60 (Sr-HSP10, Sr-HSP60). HSPs are well known as chaperones involved in stress responses of cells, but recent studies suggest additional roles of small HSPs for parasite biology including immune modulation. To characterize Sr-HSP10, we cloned its full-length cDNA, analysed the genomic organization, tested its presumptive role as interaction partner of Sr-HSP60, studied its transcription in the parasite, and expressed the protein to test its immune responses. The Sr-HSP10 protein is highly homologous to that of the human pathogen S. stercoralis with only eight amino acid substitutions. Analysis of the genomic organisation of the Sr-HSP10 locus revealed that the gene is linked head-to-head to the gene encoding Sr-HSP60, and both share a bidirectional promoter. RT-PCR experiments indicated potential independent expression of the Sr-HSPs genes. In situ-hybridisation results demonstrate Sr-HSP10 transcription in the gut area. Mammalian and yeast two-hybrid assays show dimerisation of Sr-HSP10, but no binding to recombinant Sr-HSP60. Cell binding experiments show binding to rat intestinal epithelial cells. Immunisation experiments finally revealed a strong immunogenicity of Sr-HSP10 and provided evidence for a role in regulating the host-parasite interaction.

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Review of Fatal Cases from 2007 Dengue Outbreak in Puerto Rico

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Background: In 2007, Puerto Rico had a large dengue outbreak with simultaneous transmission of all four dengue viruses, only the second time this has been documented on the island. This outbreak was associated with an unusually high proportion of severe cases. **Methods**: Fatal cases from 2007 were identified using data from the island-wide, laboratory-based passive dengue surveillance system (PDSS) and death certificates. CDC physicians reviewed medical records from dengue laboratory-confirmed fatal cases using a standardized instrument. **Results**: Of 10,508 suspected dengue cases reported to the PDSS, 5,517 (52.5%) were hospitalized, 3,340 had a hemorrhagic manifestation and 40 had a fatal outcome. Of the 40 fatal cases, 11 were laboratory-confirmed as dengue. Laboratory-confirmed fatal cases were older (median, 26.2 years) than laboratory-confirmed non-fatal cases (median, 20.7 years). Five of 11 (45.5%) laboratory-confirmed patients were ≤15 years old. Five of the six adult fatal patients had significant co-morbidities including asthma, diabetes, and hypertension. Nine of the laboratory-confirmed cases were admitted to a hospital; two presented dead on arrival. Seven of 11 (63.6%) had previously sought medical care prior to their terminal visit; three of them had 2 or more prior visits. Most common diagnoses at these prior visits included upper respiratory tract infection and abdominal pain with vomiting. Only one patient was initially identified as having a dengue-like syndrome. Warning signs of severe dengue, including persistent vomiting, abdominal pain, and narrow pulse pressure were documented in 3 of 7 of patients during these initial visits. Of the 9 admitted patients,

5 died on the day of admission (median length of stay: 26 hours). **Conclusions**: Our review suggests that there may be gaps in provider knowledge concerning dengue identification, diagnosis, and clinical management. Further physician education may be warranted.

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Genome-wide PfEMP1 arrays reveal novel domain-host receptor interacting pairs and naturally acquired neutralizing antibodies

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PfEMP1 proteins comprise a family of variant antigens that appear on the surface of *P. falciparum*-infected erythrocytes (IEs) and bind to multiple host receptors. Their large size generally precludes heterologous expression in functional form, and therefore only a limited number of single domains from various genomes have been expressed and tested for adhesion previously. Using a mammalian cell system, we expressed the entire repertoire of PfEMP1 domains from 3D7 strain genome. Most DBL-CIDR domain combinations were expressed as single structure-functional units. Using BioPlex technology, we tested adhesion activities of this PfEMP1 domain array and identified novel PfEMP1 domain-host receptor interacting pairs. We also tested sera from children and adults residing in malaria endemic areas, both for immunoreactivity to the repertoire of PfEMP1 domains and for functional inhibition of binding interactions. Results of these experiments will be presented. These data contribute further into understanding of PfEMP1-host receptors interactions and their relevance to development of protective immune response against malaria.

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***Plasmodium falciparum* resistance to antimalarial drugs in Papua New Guinea: Evaluation of a community-based approach for the molecular monitoring of resistance**

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Background
Molecular monitoring of parasite resistance has become an important complementary tool in establishing rational antimalarial drug policies. Community surveys provide a representative sample of the parasite population and can be carried out more rapidly than accrual of samples from clinical cases, but it is not known whether the frequencies of genetic resistance markers in clinical cases differ from those in the overall population, or whether such community surveys can provide good predictions of treatment failure rates.
Methods
Between 2003 and 2005, *in vivo* drug efficacy of amodiaquine or chloroquine plus sulphadoxine-pyrimethamine was determined at three sites in Papua New Guinea. The genetic drug resistance profile (i.e., 33 single nucleotide polymorphisms in *P. falciparum crt*, *mdr1*, *dhfr*, *dhps*, and *ATPase6* was concurrently assessed in community samples collected in the catchment areas of the respective health facilities by using a DNA microarray-based method. Mutant allele and haplotype frequencies were determined and their relationship with treatment failure rates at each site in each year was investigated.
Results
PCR-corrected *in vivo* treatment failure rates were between 12% and 28% and varied by site and year with variable longitudinal trends. In the community samples, the frequencies of mutations in *pfcrt* and *pfmdr1* were high and did not show significant changes over time. Mutant allele frequencies in *pfdhfr* were moderate and those in *pfdhps* were low. No mutations were detected in *pfATPase6*. There was much more variation between sites than temporal, within-site, variation in allele and haplotype frequencies. This variation did not correlate well with treatment failure rates. Allele and haplotype frequencies were very similar in clinical and community samples from the same site.
Conclusions
The frequencies of genetic antimalarial resistance markers appear to be very similar in community and clinical samples, but cannot be used to make precise predictions of treatment failure rates. Thus indicators based on molecular data have to be considered with caution and interpreted in the local context, especially with regard to prior drug usage and level of pre-existing immunity. Testing community samples for molecular drug resistance markers is a complementary tool that should help decision-making for the best treatment options and appropriate potential alternatives.

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The TLR9 agonist CpG fails to enhance the acquisition of *Plasmodium falciparum*-specific memory B cells in semi-immune adults in Mali

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Antibodies play a key role in controlling blood stage malaria infections, and an effective blood stage malaria vaccine will likely require that it induce vaccine-specific memory B cells (MBCs). Our previous studies showed that the addition of the TLR9 agonist CpG to *Plasmodium falciparum* protein subunit vaccines greatly increased their efficacy in inducing MBCs in nonimmune U.S. volunteers. Here we show that in contrast the same CpG-containing malaria vaccine did not enhance the acquisition of MBCs in semi-immune adults living in Mali. Understanding the molecular basis of this apparent refractoriness to TLR9 agonist will be of significant interest in vaccine design.

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Alpha-tocopherol transfer protein disruption confers resistance to malarial infection

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Various factors impact the severity of malaria, including the nutritional status of the host; vitamin E, an intra and extracellular antioxidant, is one such nutrient whose absence was shown previously to negatively affect *Plasmodium* development. However, mechanisms of this *Plasmodium* inhibition, in addition to means by which to exploit this finding as a therapeutic strategy, remain unclear. We demonstrate that inhibition of α-tocopherol transfer protein (α-TTP), a determinant of vitamin E concentration in circulation, confers resistance to malarial infection as a result of oxidative damage to the parasites. Furthermore, these results suggest that inhibition of α-TTP activity in the liver may be a useful strategy in the prevention and treatment of protozoan infections.

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Cardiac Conduction Safety and Pharmacokinetics of the Coadministration of Artemether-Lumefantrine and Lopinavir/ritonavir in HIV-infected Ugandan Adults

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Background
Our study aimed at assessing the cardiac conduction safety and the pharmacokinetics of the co-administration of the CYP3A4 inhibitor lopinavir/ritonavir (LPV/r) and the CYP3A4 substrate artemether-lumefantrine (AL) in HIV-infected Ugandans.
Methods
Open-label safety and pharmacokinetic study of HIV-positive adults administered single-dose AL (80/400mg) alone or with LPV/r (400/100mg) based antiretroviral therapy (ART). Electrocardiograph (ECG) monitoring was performed and serial blood samples were assayed for artemether, dihydroartemisinin, and lumefantrine.
Results
Thirty-two patients were enrolled; 16 taking LPV/r -based ART and 16 ART naïve, ineligible for ART. All took single dose AL alone or with LPV/r. Patients on LPV/r had higher hemoglobin (14 vs 12.2; p= 0.003), lower viral load (<400 vs 26,756; p= <0.01) and body mass index (21 vs 25; p= 0.06). No serious adverse events were observed. ECG parameters in milliseconds remained within normal limits. Mean QRS-complex duration and QTc interval were higher in the LPV/r arm (87.4 vs 82.8; p=0.06) and (421 vs 404 p= 0.03). Mean PR-interval was lower in the LPV/r arm (154 vs 169; p=0.02). Heart-rate was not different in the two arms. Mean change in QTc from baseline was greater for the ART naïve arm (6.7 vs -0.8; p= 0.17). None had mean QTc above upper limit of normal (470ms). QTc measurements did not change significantly over 72 hours, however, they were higher in the LPV/r arm at 24 (424 vs 406; p=0.02) and 72 hours (424 vs 408; p=0.004) post AL intake.
Conclusions

Co-administration of single dose AL with LPV/r was safe. Patients taking LPV/r had higher QTc interval. Safety of LPV/r with standard six-dose AL regimens should be investigated.

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Long-term monitoring of multidrug-resistant falciparum malaria in Thailand.

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Malaria, one of the most common parasitic diseases, remains a major public health problem in many tropical and subtropical countries. In Thailand, malaria is endemic along its country's borders, especially the Thai-Myanmar and Thai-Cambodia borders. Multidrug-resistant falciparum malaria continues to be a major problem in these border areas. Monitoring of multidrug-resistant malaria enables Thailand to be able to forecast the trends in areas prone to resistance and to implement management strategies such as dosage adjustment and change in drug regimens to stop or delay the resistance. In our on-going monitoring of multidrug-resistant malaria in Thailand, we conducted 28 and 42 days therapeutic studies according to the WHO protocol in 9 provinces along Thailand's borders in 1997 and 2008. Three regimens of the studies were 1) 15 mg/kg of mefloquine single dose (M3), 2) 15 mg/kg of mefloquine combined with 12mg/kg of artesunate (M3A), and 3) 25 mg/kg of mefloquine combined with 12 mg/kg of artesunate (M5A). Results revealed that high treatment failures occurred in Trat, Tak, and Ranong provinces. These results also warn us on a possibility of ACT resistance spreading to other areas. In conclusion, Thailand urgently needs to confirm parasite resistance in these areas by investigating parasite drug sensitivity, molecular marker, and pharmacokinetics so as to prevent the spread of multidrug-resistant malaria. .

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***Leishmania major*: Anti-leishmanial acrtivity of *Nuphar lutea* extract mediated by the activation of transcription factor NF-κB**

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Leishmaniasis caused by the obligate intracellular protozoan parasite of the genus *Leishmania* is still considered a major health problem worldwide. The number of effective drugs available against the disease (pentostam, pentamidine, amphotericin B, paromomycin, miltefosine) is still very limited. Also, many of the available drugs are toxic and in certain cases parasite drug resistance is developed. The development of new, cheap anti-leishmanial treatment is necessary for the treatment and control of the disease. In our recent study, 41 plant extracts belonging to 28 different families were examined, of which 9 showed leishmanicidal effect against *L. major* promastigotes and amastigotes *in vitro*. None of these plants totally eliminated the intracellular amastigotes, except the *Nuphar lutea* extract and its partially purified alkaloids fraction (NUP), that was almost as effective *in vitro* as paromomycin - the gold standard drug against the disease. Here we report the effect of NUP on NF-κB expression and determined its mechanism of toxicity against *Leishmania major* in C3H mice peritoneal macrophages. NUP was found to be a mixture of thermo-stable dimeric sesquiterpene thioalkaloids containing mainly thionupharidines and/or thionuphlutidines. A total elimination of the intracellular amastigotes, with no toxicity to the macrophages, was achieved with NUP at 0.17μg/ml (IC_{50} 0.087±0.003 μg/ml), within 3 days of treatment. The anti-leishmanial activity was shown to be mediated through the activation of NF-κB and increased iNOS production. *N. lutea* NUP was also shown to act as an anti-oxidant, almost completely inhibiting the macrophage respiratory burst activity. However, no elevated lysozyme (EC3.2.1.17) or β-galactosidase (EC3.2.1.23) activities were demonstrated in macrophages treated with NUP. The present study suggests that activation of NF-κB in macrophages by *N. lutea* might be of a potential source of anti-leishmanial compounds.

2901

A Sentinel Unit and Reference Center for Malaria in an endemic area of Dengue in Brazil

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The acute febrile disease clinic of Fiocruz is an outpatient service in a network of medical assistance, epidemiologic and reference laboratories able to provide differential diagnosis to dengue fever. It acts as a sentinel center of tropical diseases in Rio de Janeiro, and for malaria in the Extra-Amazon region. The aim of this presentation is to show the principal causes of febrile cases seen from January 2005 to January 2008 in our clinic. We targeted adults reporting fever up to 10 days from the date of first consultation. Laboratorial diagnosis is performed in all patients' sera for dengue IgM antibodies detection by Elisa-capture (PanBio, Australia). The search for malaria is routine for any febrile patient coming from endemic areas. Among 511 febrile cases, dengue was by far the most prevalent, 37.4%, malaria 4.7%, acute CMV 4%, toxoplasmosis 3%, leptospirosis 1.4%, viral hepatitis 1.2%, rubella 1.6%, among others. There was no conclusive diagnosis in 43.3% of them. For malaria, Amazon was the most important region of origin, for the cases of *P.* vivax

infections, and Africa for the *P. falciparum* cases. Two autochthonous cases (*P. vivax*) were seen coming from the Atlantic Forest, which is located outside the Amazonian region that concentrates more than 99% of malaria in Brazil. The positive and negative predictive value of clinical diagnosis for dengue was 56% and 90% respectively. Among those with clinical suspicion of malaria, 58.5% had laboratory diagnostic confirmation. The negative predictive value of clinical suspicion for malaria was 100%. We believe our systematic approach increases the chances of recognizing infectious disease. Indeed, it is important to include in the surveillance strategy the laboratorial diagnosis of malaria that, if misdiagnosed, may lead to fatal consequences. Little is known about the transmission chain of indigenous malaria in the Atlantic forest (in the state of Rio de Janeiro). As monkeys of the genera *Allouata* and *Cebus* live in this forest in the Rio de Janeiro and São Paulo states, where *Kerteszia cruzii* and *Ker. bellator* are also present and could be responsible for both human and simian malaria transmission, the possibility of a close relationship between simian and autochthonous human malaria in extra-Amazonian region is to be considered. Migration and international travel require personnel with specific expertise, highlighting once more the importance of the specialty of Infectious Diseases.

2902

Development of antibody-dependent enhancement assay for dengue virus using stable BHK-21 cell lines expressing FcγRIIA

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Dengue virus (DENV) causes a wide range of symptoms, from mild febrile illness, dengue fever (DF), to severe life threatening illness, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Subneutralizing concentrations of antibody to DENV enhance DENV infection of FcγR positive cells. This phenomenon is known as antibody-dependent enhancement (ADE). ADE is considered to be a risk factor for DHF and DSS. To develop an ADE assay for DENV, two stable BHK-21 cell lines that express FcγRIIA were established. The FcγRIIA-expressing BHK-21 cell lines were used in ADE assay with monoclonal antibody (4G2) to DENV, and DENV antibody-positive human sera. Virus growth was quantified directly in FcγRIIA-expressing BHK-21 cells by standard plaque assay procedure. ADE was detected with monoclonal antibody (4G2) to DENV. ADE was also detected with DENV antibody-positive human sera, but not with DENV antibody-negative human sera. Neutralizing antibody titers towards to DENV in the presence of FcγRIIA was also examined, and discrepancy in DENV neutralizing titers between FcγRIIA-expressing and non-expressing cell lines were observed. The new ADE assay using FcγR-IIA expressing BHK-21 cells is simple and practical, and could define the effect of ADE and DENV neutralization titers.

2904

A longitudinal analysis of the effect of mass drug administration with diethylcarbamazine (DEC) on lymphedema-specific filarial morbidity in Léogane, Haiti

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The twin goals of the global lymphatic filariasis (LF) elimination program are to interrupt LF transmission through mass drug administration (MDA) and to manage and prevent disability for affected individuals through morbidity control programs. However, the effect of MDA with diethylcarbamazine (DEC) on lymphedema-specific filarial morbidity is unknown. A cohort of 175 lymphedema patients enrolled in a lymphedema management study from 1995-1998 in Léogane, Haiti was targeted for follow-up in 2008. MDA began in Léogane in the year 2000. From this cohort, 117 (67%) patients were evaluated in 2008; 102 (87.2%) who received DEC through MDA and 15 (12.8%) who never received DEC. Cross-sectional and prospective cohort data from 8 time points over a 13 year period were compiled to evaluate lymphedema progression, and acute episodes of adenolymphangitis (ADLA). Quality of life was assessed retrospectively in 2008.
No difference in lymphedema progression between those who received or did not receive DEC was found on measures of foot circumference (P=0.24), ankle circumference (P=0.87), leg circumference (P=0.46), volume displacement of the leg (P=0.09), stage of lymphedema (P=0.93) or frequency of ADLA episodes (P=0.57) controlling for significant covariates. Nevertheless, patients who received DEC reported improvement in four areas of quality of life related to lymphedema ($P\leq0.01$). Rates of ADLA per 12 month period were greater after the year 2000 among both groups ($P<0.01$). Decreases in foot and ankle circumference and ADLA episodes were observed during the 1995-1998 lymphedema management study ($P\leq0.01$).
This is the first longitudinal analysis of the effect of MDA with DEC on lymphedema-specific filarial morbidity. Despite lack of improvement in lymphedema progression or ADLA episodes with DEC, lymphedema patients who take DEC through MDA report improvements in quality of life. Increasing rates of ADLA after 2000 may result from continued high LF transmission in the area as well as the cessation of the lymphedema management study in 1998. Furthermore, these results highlight the positive impact of lymphedema management programs on measures of lymphedema-specific morbidity. LF elimination programs should prioritize both lymphedema management and MDA to achieve their goal of reducing LF-related disability.

2905

The dengue virus type 4 vaccine candidate rDEN4Δ30-4995 is highly attenuated, safe, and immunogenic in healthy adult volunteers

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rDEN4Δ30-4995 is a live attenuated dengue virus type 4 (DEN4) vaccine candidate that was designed as a further attenuated derivative of the rDEN4Δ30 vaccine virus. In a previous study, subcutaneous administration of 10^5 PFU of rDEN4Δ30 was associated with mild transient serum alanine aminotransferase (ALT) elevations in 5/20 vaccinees, and 10/20 developed an asymptomatic maculopapular rash. In the current study, 28 healthy adult volunteers were randomized to receive a single dose of 10^5 PFU of rDEN4Δ30-4995 (20) or placebo (8). rDEN4Δ30-4995 was safe, well-tolerated, and immunogenic. However, in contrast to rDEN4Δ30 vaccinees, 17/20 rDEN4Δ30-4995 vaccinees developed an asymptomatic localized erythematous rash at the injection site. Mild and transient elevations of serum ALT were observed in 2/20 of the rDEN4Δ30-4995 vaccinees, and 2/20 vaccinees developed an asymptomatic maculopapular rash similar to that seen with rDEN4Δ30. None of the rDEN4Δ30-4995 volunteers became viremic, yet 95% developed a four-fold rise in neutralizing antibody titers. Thus rDEN4Δ30-4995 was demonstrated to be safe, highly attenuated, and immunogenic while exhibiting mild local reactogenicity.

2906

Intense inflammatory response and high mortality rate are associated with dystrophin reduction in *Trypanosoma cruzi*-infected mice.

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Background. Heart failure is an important cause of morbidity and mortality in both acute and chronic phases of Chagas' disease. In addition to functional alterations, heart failure has a structural basis as well. Taking into account that the contractile machinery inside the myocyte must remain intimately connected with the membrane and extracellular matrix, association provided by the dystrophin glycoprotein complex (DGC), this study was carried out to test the hypothesis that acutely infected murine hearts present changes of dystrophin expression.
Methods. C57Bl/6 mice were infected with Y strain of *Trypanosoma cruzi*. The animals were killed 9, 14, 20, 26 and 32 days after infection and parasitemia and mortality rate were observed. Histological analysis and immunohistochemistry for *T. cruzi* were performed. Immunofluorescence staining and western blotting were performed for dystrophin.
Results. Mice displayed a parasitemia peak at day 9 and the mortality rate reached a peak on day 20. The histological findings demonstrated that inflammatory infiltrate was present at day 14, peaked at day 20, and became less intense at day 26. The immunohistochemistry for *T. cruzi* showed parasite persistence up to 20 days after infection and then it became rare at 26 day. Different levels of protein loss were observed with immunofluorescence. Dystrophin was focally reduced at day 14 and completely lost 20 days after infection. A correlation between the amount of dystrophin and mortality rate was observed. The peak of mortality was correlated with the complete focal loss of dystrophin. Western blotting analysis confirmed the decreased expression of this structural protein.
Conclusions. Our results lend support to the hypothesis that changes in cytoskeletal proteins and, in particular, dystrophin could represent the partial loss of the mechanical binding of the entire array of myofibrils to the extracellular matrix, thus providing a final common pathway for contractile dysfunction and sudden death in mice experimentally-infected with *Trypanosoma cruzi.*

2907

Vitellogenin promoter driven transgenic Rel2 - mediated Plasmodium resistance in Anopheles stephensi

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The innate immunity of mosquitoes is the primary line of defense against the malaria parasite Plasmodium and other microbes. It mainly comprises of the TOLL and IMD pathways where the two NF-kappa B-like transcription factors, Rel1 and Rel2 translocates to the nucleus and activates the transcription of several antimicrobial peptides and many other effector genes. IMD pathway is the major player in regulating resistance of several Anopheles species to numerous malaria parasites and is more likely appealing for the generation of genetically modified mosquitoes that are resistant to Plasmodium species. The Rel2 gene (orthologous to Drosophila Relish) of the malaria vector A. gambiae, has been shown to control the expression of several immune genes (LRIM1, CLIPB14, KIN1, FBN etc) or antimicrobial peptides like Cecropin, Gambicin and also regulate the bacterial and Plasmodium infections. The A. gambiae Rel2-S (Rel2 short form lacking the inhibitory ankyrin repeats and death domain) transcript has been cloned under the A.

gambiae vitellogenin promoter to generate blood-fed inducible Rel2 transgenic mosquitoes (fat-body specific) in Anopheles stephensi. We have observed a decreased Plasmodium falciparm infection phenotype (~50% lower oocyst intensity); upon activation of Rel2 transgene (and the IMD pathway) after feeding on infectious blood-meal. We have furthermore explored the regulatory role of Rel2 in mosquito innate immunity in mosquito cell-lines (Sua 5B and MSQ43), and the induction of Rel2 up-regulates several effector molecules like Cecropin, Defensin, Gambicin and FBN9 genes by more than two fold. Studies are ongoing to look at the regulation of other immune genes of the IMD pathway and the effector molecules upon Rel2 activation, after challenge with other parasites (Plasmodium berghei) and various bacterial isolates and the corresponding mortality will also be monitored. Additionally, we plan to look at the fitness parameters; the longevity and fecundity of the Rel2 transgenic mosquito lines.

2908

Screening and treatment of Cystic Echinococcosis in Yushu, Tibetan Plateau, Qinghai.
Preliminary results from a pilot study.

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In 2003, doctors volunteering for Rokpa International (RI), an NGO with headquarters in Zuerich, Switzerland and branches in 18 countries, initiated a collaborative humanitarian project in Yushu, Qinghai, Oriental Tibetan Plateau, PRC, offering free medical assistance to nomadic people living in the area. Patients were visited in a basic outpatient facility of Yushu Medical School and Orphanage, RI.
Beginning in 2007, free ultrasound (US) examinations were also offered, and an informal screening of 578 individuals found a 6,74 % (39 pt, m/f 12/27, mean age 42.79±14.50 y) infection rate of cystic echinococcosis (CE). Many patients were children and young adults with active cysts, which suggests a high parasitic pressure and the need for urgent control measures. 28% (11, 4m/7f, m.a. 30.45±15.04 y) of patients had relapses from previous surgeries.
Given the high infection rate, a more structured screening was set up in 2009 after a formal agreement with local surgical and radiological staff from "Yushu People's Hospital" that included treatment and follow-up of infected patients.
Four hundred eighty new patients (that is, excluding patients that had been screened in 2007) were scanned, with an infection rate of 5.62% (27 pt, m/f 15/12, m.a.42.59±11.39 y) and similar demographics (relapse of previous surgery in 37.3 %, 10 pt, 2 m/8 f).
Overall, 1058 patients were screened over two years, with a 6.23% (66 pt) CE infection rate. Of those, 39 (59%) had one ore more active cysts (CE1, CE2) 11 (16.6%) had transitional (CE3a/b) cysts and 29 (43.9%) had inactive (CE4,CE5) cysts. (The total number of cysts is greater than the number of patients because some of them harbored multiple cysts at different stages). Twenty-one patients (46.96%) had a post-surgical relapse. Selected cases (1 in 2007 and 3 in 2009) were treated with PAIR and catheterization plus oral albendazole.
Our findings warrant the continuation of this collaborative effort that should be expanded to include US training of local doctors in diagnosis and PAIR of abdominal CE and the initiation of control programs for CE.

2913

Ready-to-use-supplementary foods and the prevention of wasting, stunting and mortality among children aged 6 to 36 mo in Niger: a non-randomized intervention study

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Ready-to-use-therapeutic foods (RUTF) have been shown effective in the treatment of severe wasting in children and have transformed the treatment of child malnutrition with the provision of community-based care and treatment. The acceptability and effectiveness of RUTF have led to the development of new, targeted ready-to-use spreads, including ready-to-use supplementary foods (RUSF), but the effectiveness of RUSF relative to RUTF in the prevention of malnutrition in children has not been evaluated.
In a non-randomized intervention study in Maradi, Niger, we compared the incidence of wasting (weight-for-height (WHZ) < -2), severe wasting (WHZ < -3), stunting (height-for-age (HAZ) < -2), and severe stunting (HAZ < -3) according to the World Health Organization growth standards, and mortality among children aged 6 to 36 mo receiving preventive supplementation with either RUSF (247 kcal/d for 6 mo) or RUTF (500 kcal/d for 4 mo).
We found the effectiveness of the RUSF strategy to depend on receipt of a previous preventive intervention. In villages where a preventive supplementation program was previously implemented, the RUSF strategy was associated with a 46% (95% CI: 6% to 69%) and 59% (95% CI: 17% to 80%) reduction in wasting and severe wasting, respectively. In contrast, in villages where the previous intervention was not implemented, we found no difference in the incidence of wasting or severe wasting by supplementation strategy. Compared to the RUTF strategy, the RUSF strategy was associated with a 19% (95% CI: 0% to 34%) reduction in stunting overall.
Our study suggests that the RUSF strategy, providing lower energy for longer duration, reduced the risk of wasting and severe wasting

where a preventive intervention was previously implemented and of stunting overall but did not appreciably differ from the RUTF strategy in villages that did not receive the previous intervention. The choice of dose and duration of supplementation will need to be guided by the effectiveness and cost-effectiveness of the overall program according to the context.

2914

Prevalence of *Plasmodium falciparum* carrying chloroquine resistant mutant pfcrt genotypes in the South Pacific islands of Vanuatu and the Solomon Islands

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Malaria drug resistance in the Solomon Islands and Vanuatu is poorly understood. It is believed that the prevalence of drug resistance may be in flux as first line treatments are becoming less effective. To assess the parasite drug resistance profiles, we have analysed the prevalence of parasites carrying specific polymorphisms within the Pfcrt gene in 50 and 101 *P. falciparum* samples collected during recent epidemiology surveys conducted in Vanuatu (Tafea Province) and the Solomon Islands (Temotu Province) respectively. All samples from Vanuatu contained the K76T polymorphism and most parasites have the SMNT haplotype at codons 72, 74, 75 and 76, identical to patterns observed in parasites collected from Papua New Guinea. All Solomon Island samples currently analysed also contain the K76T mutation. Ongoing efforts will examine other sequence polymorphisms in the entire Pfcrt. Approximately 30% of the collected samples contain both *P. falciparum* and *P. vivax*. We will examine Pfcrt isolated from mono and mixed infections to determine if a relationship between multiple infections and Pfcrt polymorphisms exist. Understanding these drug sensitivity patterns may assist in the malaria eradication efforts currently underway in the South Pacific.

2915

Household Hygiene Variables and Its Impact on the Risk of Campylobacter Diarrhea among Children of Rural Egyptian Villages

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Background
Campylobacter sp. is one of the most frequently isolated bacteria from stools of infants with diarrhea in developing countries. Among other factors including the host and agent, environmental factors including domestic sanitation and poor hygienic conditions are thought to influence risk of Campylobacteriosis in these endemic settings. The present study aims to identify the impact of various hygiene variables on increased risk of Campylobacter associated diarrhea among children <2 years of age in rural Egyptian villages.
Methods
A prospective birth cohort study of 348 infants was conducted from January 2004 to April 2007 in five villages of the Nile Delta region. Neonates were enrolled at birth and followed up to 24 months of age. Children were visited twice a week to survey for acute diarrhea. A detailed observational household hygiene survey was completed in-house approximately every six months during the two-year follow-up period. The hygiene questionnaire consisted of ten questions and covered the domestic environmental conditions of the home and hygiene characteristics of sleeping room, eating room, cooking room, garbage containers, previously prepared food, washing facilities, bathroom and bathing facilities, water sources and containers, and flies in the house. Adjusted Incidence Rate Ratios (IRR) of hygiene variables were calculated for the primary outcome of Campylobacter-specific diarrhea illness rates.

Results
The risk of Campylobacter associated diarrhea significantly increased with several household hygiene variables. Presence of human or animal feces around the bathroom (IRR 2.48, $p<0.001$), non-daily washing of garbage containers (IRR 1.93, $p<0.05$), location of the bathroom inside the house (IRR 2.39, $p<0.05$), absence of barriers to keep birds and animals out eating rooms (IRR 1.51, $p<0.05$), and cooking rooms (IRR 1.70, $p<0.05$), and drain of latrine open to the environment (IRR 1.53, $p<0.05$) all increased a child's risk of Campylobacter-diarrhea in the household.
Conclusion
Factors related to household hygiene and construction appears to be associated with an increased risk of Campylobacter-diarrhea disease. Further study is warranted to evaluate these factors on all-cause and other pathogen-specific causes of diarrhea. These data are important in highlighting some potentially modifiable factors which could reduce the burden of disease in resource-poor settings.

2916

In vitro pharmacodynamic and cytocidal activity of 1,2,3,4-tetrahydroacridone and 4(1H)-quinolone antimalarials

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4(1*H*)-quinolones (4Qs), and 1,2,3,4-tetrahydroacridones (THAs) are novel antimalarial chemotypes that likely target the functions of the parasite's mitochondria. 4Qs are known causal prophylactic (kill growing liver stage parasites) and potent erythrocytic stage agents in avian malaria models, but not against malaria parasites in mammals (A. C. Casey, *J. Med. Chem.* 1974, *17*, 255). Initial observations by us uncovered erythrocytic stage activity on *P. falciparum in vitro* led us to optimize these leads in quantitative structure property relationship studies at the University of South Florida. Herein, we report *in vitro* pharmacodynamic studies on selected derivatives from the THA (RMMC_93) and 4Q series (RMMC_95 and RMMC_105). Interestingly, these chemotypes manifested excellent activity versus either atovaquone susceptible or resistant parasites and even showed more than 10,000-fold specificity towards parasites than mammalian cell lines. The three tested compounds manifested a similar trend in the rapidity of antimalarial action in W2 as well as 3D7 *P. falciparum*. The inhibition of nucleic acid synthesis starts from 24 to 36 hours depending on the concentration exposed, with the most significant inhibition observed with highest concentration tested (120 ng/ml). The 50% growth inhibition time (TI_{50}) was similar irrespective of concentration to which the parasite was exposed. In comparison, TI_{90} was similar with that of atovaquone (10 ng/ml) for concentrations 60 ng/ml and above. The rate of antimalarial action of these RMMC compounds was similar to the rapidity of action of atovaquone. Importantly the THA and two 4Qs displayed not only remarkable erythrocytic stage activity, but resistance could not be selected for any of these drugs in an ARMD phenotype assay. These studies suggest the THA and 4Q derivative have outstanding potential as new antimalarial drugs with nM potency for blood and liver stages of malaria.

2917

Isolation of yellow fever virus from Sabethes (sabethes) albiprivus from Misiones, Argentina

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By the end of 2008 and beginning of 2009 an epizootic of yellow fever (YF) killed many howler monkeys (Allouatta sp.) in Misiones province, NE of Argentina. Mosquitoes were captured in the same geographic region. One strain of YF virus was isolated in cell culture from 1 out of 20 pools of Sabethes albiprivus tested. This is the first isolation of YF virus from mosquitoes in Argentina; it is also the first YF virus isolation reported from Sabethes albiprivus.
In Argentina, during the summer of 2007-2008, a selvatic outbreak of YF affected monkeys and humans, after 50 years without viral activity detected in Argentina. In December 2008/January 2009, a high mortality rate of monkeys close to Posadas city (Misiones province) was observed. The objective of the present study was to investigate the circulation of YF virus in mosquitoes that could be implicated in the selvatic transmission of YF virus in Argentina.
In January 2009, field studies at the subtropical rain forest surrounding Posadas city were conducted. Mosquitoes were captured from human bites and by using CDC traps. Insects were kept in liquid nitrogen until transported to the laboratory. Specimens were sorted according to method of collection, location, date of capture and genus. Supernatant of mosquitoes pool homogenates was inoculated into Vero and C6/36 cells for virus isolation and RT-PCR for flavivirus studies. Virus isolates were identified by imnumoflouescence using monoclonal antibodies and by RT-PCR.
Out of 506 mosquitoes captured, 54 belonged to the species Sa albiprivus. One YF virus strain was isolated from one out of 20 pools (54 mosquitoes) of Sa. albiprivus prepared. Virus isolation and RT-PCR studies of the remaining mosquitoes species captured are ongoing.
The present study reports the first YF virus isolation from mosquitoes in Argentina; it is also the first YF virus isolation reported from Sa. albiprivus. These results contribute to the knowledge of the selvatic cycle of YF virus in Argentina.

2918

Arterolane, a new synthetic peroxide, in combination with PQP is effective when compared to artemether-lumefantrine: A Phase II study in umcomplicated *P. falciparum* malaria

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A randomized open label, multicenter study comparing arterolane maleate and piperaquine phosphate (AM+PQP) to artemether-lumefantrine (A+L; Coartem®) administered for 3 consecutive days was conducted in 240 patients (allocated in 2:1 ratio). The study provided 229 evaluable patients between 13 to 65 years of age in Thailand and India.
In ITT population, the PCR corrected ACPR on Day 28 was 94.4% for AM+PQP and 96.3% for A+L (Odds ratio 0.670, 95% CI,

0.177-2.539 between the two regimens). In PP population, the PCR corrected ACPR on Day 28 for AM+PQP was 100% as compared to 98.7% in A+L. In ITT population, in either of the two regimens, the median PCT was 30 hours (25 - 75 percentile 23 -40 hours for AM+PQP & 23-42 hrs for A+L) and median FCT was 24 hrs. The PCR uncorrected ACPR values were the same as PCR corrected ACPR on Day 28. In both the treatment arms, the median values of gametocyte counts decreased till day 2, after which the values increased on Day 7 with a steady decline on Days 14, 21 and 28.
AM+PQP treatment was well tolerated by the patients. The overall incidence of treatment emergent AEs was similar in the 2 treatment groups (86.3% in AM+PQP vs 85% in A+L). Two SAEs were reported, one with AM+PQP (hyperventilation syndrome) and the other with A+L (malena & inability to pass urine), both were 'not related' to the study drugs. Other AEs reported were mild to moderate in intensity, and had resolved by the end of the study. The incidences of AEs related to the gastrointestinal systems such as vomiting, dyspepsia and pain in upper abdomen were similar in both treatment arms (6.3%). 68.3% of AEs were related to out of range laboratory values. Decrease in hemoglobin was comparable in both the treatment groups. No deaths were reported.
The pharmacokinetics of arterolane and piperaquine were characterised. Pharmacokinetic results indicate that the C_{max}, AUC_{0-24} and AUC_{4pts} of arterolane on Day 2 were 1.24, 1.34 and 1.36 folds, respectively of that observed on Day 0. The higher exposure values on Day 2 are attributed to the patients becoming aparasitemic. Piperaquine exposure increased on Day 2 following multiple dosing as compared to single dose exposure. The mean increase in C_{max}, AUC_{0-24} and AUC_{4pts} of piperaquine on Day 2 was 2.43, 3.31 and 3.19 folds, respectively. The accumulation on multiple dosing of piperaquine is expected based on the long elimination half life.
The efficacy and safety of AM+PQP is similar to A+L and will be further evaluated in Phase III trials.

2920

Carnivore seroprevalence as sentinel for human plague cases

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Coyotes and other carnivores are susceptible to infection by *Yersinia pestis*, the etiological agent of plague, but are not thought to play a role in plague maintenance. Carnivore serosurveys have been shown to aid in identifying localities associated with plague epizootics, but these data have seldom been used to identify inter-annual variation in risk. Moreover, although it is often cited that carnivores serve as good sentinels for plague activity and thus useful as indicators of human risk, to our knowledge, the association between carnivore seroprevalence and human case occurrence has never been evaluated empirically. In this study, we tested for an association between coyote seroprevalence and human case occurrence in four Arizona, USA counties from 1974 through 1998. Positive passive hemagglutination and inhibition (PHA) test titers above 1:256 (n=354 of 2,276 samples) were considered to be indicative of recent exposure to plague bacteria. Human case counts (n=52) were downloaded from the Arizona Department of Health Services (http://azdhs.gov/phs/oids/vector/plague/stats.htm) for the same period and counties as the coyote data. The correlation between human cases and percent of sero-positive coyotes was positive and significant ($r=0.48$, $p=0.01$). To further explore this relationship, we dichotomized the human data at the median (1 case) and considered more than one case to be a high year and 1 or fewer cases to be a low year. High coyote years were defined as those where the percent of positive titers was greater than 10%. Of the 25 years for which there were coyote and human data, 8 years were consistently low (coyote titer <10%; human cases ≤1) and 10 years were consistently high (coyote titer >10%; human cases>1) (Fisher's Exact $p<0.05$). The remaining 7 years were mismatched with 4 years where low human cases coincided with high coyote seroprevalence and 3 years with high human cases and low coyote seroprevalence. The results of this analysis show a significant association between coyote seropositivity rates and human plague occurrence that can be useful for identifying years with an elevated risk of human exposure to *Y. pestis*.

2921

Establishment of an in vitro medium-throughput, and validation of a high-throughput screening assay to assess chemical toxicity at WRAIR.

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In the early stages of drug discovery, *in-vitro* toxicity screens are inexpensive, rapid, and useful tools for comparing and deselecting compounds with greater toxicity within a chemical series, and may aid in making decisions about compound advancement for *in vivo* studies.
During the past year, we have established the methodology for routine screening of chemical entities against a rat macrophage cell line. We programmed the Biomek 2000 robotic fluid handler to accommodate the *in-vitro* toxicity screen in 96 well plates, with a capacity of several hundreds of compounds per year. In collaboration with the Chemical Information Department we have been successful in capturing, analyzing, and presenting the data in the Chemical Information System (CIS), so any investigator can access that information. In addition, we validated the protocol for the in-vitro toxicity screening of compounds in a human hepatocyte cell line. This effort supports the *in-vitro* liver assay that was recently validated and established in our Department.
Using Biomek 2000 we also validated a similar protocol in a 384-well plate format. The assay parameters (Z factor, signal/noise, signal/background ratio, and coefficient of variation) indicate that this is a robust assay which in the future will be integrated with the high throughput cell-based SYBR green assay for malaria drug screening.

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LIVER ABSCESS IN THE TROPICS: AN EXPERIENCE FROM NEPAL

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Thirty-six consecutive cases of liver abscess seen at the BP Koirala Institute of Health Sciences Hospital, Dharan, Nepal, were reviewed. Twenty-one cases were male and 15 female, with a mean age of 42 years. Twenty-four cases (66.7%) were amebic, 7 (19.4%) pyogenic, 3 (8.3%) indeterminate and 2 (5.5%) tuberculous. The most frequent clinical features included fever (88%), leukocytosis (66.7%), abnormal level of serum albumin (44.4%) and alkaline phosphatase (38.9%).
The liver abscess was single in 61.1%, multiple in 27.8%, and in 66.7% of cases the abscess was present in the right lobe of the liver. Ultrasonography was diagnostic in all cases. A positive culture of the abscess was obtained in 7 cases (19.4%). The most frequent bacteria found were *Klebsiella pneumoniae 4* (11.1%), followed by *Escherichia* coli 3 (8.3%). Two cases were due to *Mycobacterium tuberculosis* and none had malignancy. Percutaneous drainage was performed in 27 patients (75%). Mortality attributable to the abscess was 5.5%. We found percutaneous needle aspiration of liver abscess helpful in confirming diagnosis, as it provides a better bacteriological culture yield, gives a good outcome, and may uncover clinically unsuspected conditions like malignancy and tuberculosis. These two conditions should certainly be considered possible causes in our part of the world when an abscess fails to respond to standard treatment.
In developing countries like Nepal, the clinical presentation of liver abscess has not varied over time. At present, rapid diagnosis and image-guided percutaneous drainage offer a better prognosis for liver abscess.
We also recommend routine cytological examination of aspirated abscess materials, as well as stains and cultures for acid-fast bacilli.

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Endothelial activation markers associated with severe and fatal malaria in Ugandan children

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Introduction: Endothelial cell (EC) activation and dysregulation are thought to be central processes in the pathogenesis of severe malaria. The pro-inflammatory angiogenic factor Angiopoietin-2 (ANG-2) is released from activated ECs, and ANG-2 levels have been correlated with disease severity and outcome in malaria patients. The ANG-2 receptor, Tie-2, is cleaved from the surface of activated ECs, and this soluble receptor (sTie-2) has been found to augment ANG-2 activity. We hypothesized that ANG-2 and sTie-2 can distinguish between pediatric patients with uncomplicated and severe malaria and predict mortality. **Methods:** Plasma samples were obtained from children (0.5-12 years old) with uncomplicated malaria (UM, n=34), cerebral malaria (CM, n=28), and severe malarial anemia (SMA, n=36) upon presentation to hospital in Kampala, Uganda. Levels of ANG-2, sTie2, and established biomarkers of severe and fatal malaria (sICAM-1, IP10) were measured by ELISA. **Results:** Plasma levels of ANG-2 ($p<0.001$), sTie2 ($p<0.001$), and sICAM-1 ($p<0.01$) were elevated in children with CM and SMA compared to UM. ANG-2 levels were significantly higher in fatal cases of severe malaria compared to survivors ($p<0.01$), as were sICAM-1 and IP-10 ($p<0.001$). Receiver operating characteristic (ROC) curve analysis showed that ANG-2 and sTie2 effectively discriminated between UM and severe malaria cases, and ANG-2 discriminated between survivors and non-survivors (area under curve: 0.814). Based on the optimal threshold value from the ROC curve, ANG-2 predicted survival among severe malaria patients with 77% sensitivity and 76.6% specificity. Positivity for at least 2 of ANG-2, IP-10, and sICAM-1 predicted fatality with 93.8% sensitivity, 79% specificity, and a negative predictive value of 97.4%. **Conclusion:** Plasma ANG-2 and sTie2 are elevated in severe malaria, likely reflecting extensive EC activation and potentially suggesting a role for these proteins in disease pathogenesis. Moreover, combinations of biomarkers may be useful for predicting fatality among children with severe malaria.

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Spotted Fever Group Rickettsial Infection in Jakarta, Indonesia

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Although spotted fever group (SFG) rickettsial infections are widespread in Southeast Asia, there has been only one documented cluster of rickettsial spotted fever in humans in Indonesia, in the far eastern province of Papua in 2003. In 2004 we trapped 211 rodents in or near human habitations in the capital city of Jakarta and seroepidemiology showed a 57% prevalence of antibodies to SFG rickettsiae (SFGR). The high proportion of seropositive peridomestic rodents suggested that spotted fevers could be a significant

and unrecognized cause of undifferentiated febrile illness among residents of Jakarta. We therefore performed indirect ELISA for SFGR-specific antibodies on 368 previously archived human serum samples from ongoing dengue and chikungunya virus surveillance studies. We then used real-time PCR to test for rickettsial DNA in sera from human seroconverters and in ectoparasites collected from the rodents of the 2004 study. Sixty (16.3%) human sera were positive for SFGR-specific IgG, thirty of these positive samples had acute and convalescent sera available, and five (16.7%) of these met seroconversion criteria of a four-fold rise in titer. We did not detect SFGR nucleic acid in any of the seroconverters' acute blood samples. However, we found SFGR DNA in pooled ectoparasites from two (5%) of the 40 rodents tested. This report documents strong evidence for human SFGR infection in the sixth largest metropolitan area in the world. SFG rickettsial infections present with non-specific symptoms and are difficult to diagnose clinically. They can cause severe or fatal illness, but if caught early most respond well to the tetracycline class of antibiotics. SFG rickettsioses should be added to the differential diagnosis of febrile illness in Jakarta, and empiric antibiotic therapy considered by health care providers.

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BEHAVIOUR CHANGE COMMUNICATION STRATEGIES FOR MALARIA CONTAINMENT PROJECT IN CAMBODIA

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Malaria is a leading cause of mortality and morbidity in Cambodia. Despite substantial progress made over the last decade, there has been however, recent evidence of artemisinin tolerance in Western Cambodia and Eastern Thailand. A containment strategy has been developed and short term funding has been secured from the BMGF. A number of new options are being made available to the communities in and visiting the affected areas in order to prevent and seek treatment for malaria, and to change old behaviours that are conducive to the spread of tolerance. A comprehensive BCC strategy for containment has therefore been developed by the BCC Working Group which was set up with experts from CNM, implementing partners and other key stakeholders. The group held a series of meetings during which a situational analysis was undertaken, the current BCC strategies were reviewed, key containment messages, media and audiences were agreed upon and consensus was reached on the preparatory activities, materials development and assignment of responsibilities. Indicators along with targets for measuring the progress made in the implementation of the strategy were identified.
The implementation of the strategy has begun through the development, pre-testing and finalizing a 'minimum package' of containment specific IEC materials to facilitate the introduction of new containment interventions such as Mass Screening and Treatment (MSAT), active case investigation, active case detection and focal IRS. Work on the adoption of a public-private sector approach for rational drug use, to improve malaria case detection, malaria prescribing and reporting among providers, and massive BCC & community mobilization regarding treatment seeking, new drugs and adherence to treatment is ongoing. Key containment messages are being delivered through interactive radio and TV programs, Mobile Video Units, Newsletters, Standardized Package of job aids for public and private sector providers, Interpersonal communications through the vast networks of village level volunteers, National Malaria Day/Malaria Week celebrations/campaigns and Patient and Family Health Education at public health facilities by health staff.

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Insecticidal activity of sugar-insecticide solution to *Culex pipiens molestus* (Diptera: Culicidae) under the laboratory

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Insecticidal activity of sugar-insecticide solution to *Culex pipiens molestus* was evaluated under the laboratory condition. This test was composed of three bioassay. First, The susceptibility of *Culex pipiens molestus* to insecticides was evaluated under laboratory conditions using 10 insecticides currently used by the local public health centers in Korea. The insecticides included 8 pyrethroids, 1 organophosphates, and 1 carbamates. Based on LC_{50}, bifenthrin showed the highest insecticidal activity to *Cx. pipiens molestus* with the value of 0.00017 μg/[[Unsupported Character - ♀]], followed by permethrin, deltamethrin and pipimiphos-methyl with the value of 0.00060, 0.00093 and 0.00093 μg/[[Unsupported Character - ♀]], resfectively. The least insecticidal activity was obtained with etofenprox at LC_{50} of 0.00534 μg/[[Unsupported Character - ♀]]. Second, the order of susceptibility of sugar-insecticide solution was also determined by direct contact bioassay. The same kind of insecticides as those used to previous test was used to *Cx. pipiens molestus*. However, Deltamethrin show the highest insecticidal activity at LC_{50} of 0.006 mg/cm^2, followed by bifenthrin, bendiocarb and *α*-cypermethrin with the value of 0.015, 0.055 and 0.097 mg/cm^2, respectively. The least insecticidal activity was obtained with pipimiphos-methyl at LC_{50} of 0.392 mg/cm^2. It was revealed that a cause of the different insecticidal activity of tested insecticides between two bioassays was repellent activity of each insecticide to *Cx. pipiens molestus*. Bioassay on repellent activity of tested insecticides was carried out by patch test. Deltamethrin showed the lowest repellent activity (10.5% and 8.8%, 12h and 24h after treated) to *Cx. pipiens molestus,* followed by bifenthrin, bendiocarb, α-cypermethrin. Pirimiphos-methyl showed the strongest repellent activity (82.4% and 70.6%, 12h and 24h after treated) to *Cx. pipiens molestus*. Final, to see insecticidal mode of action of 3 insecticides which have high insecticidal and low repellent activity among tested insecticides, direct-non direct

contact bioassay was carried out. As a result, we could see that insecticidal activity of 3 insecticides was resulted from contact reaction.

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The Effect of Nutritional Status on the Post-treatment Prophylactic Effect of Dihydroartemisinin-Piperaquine in Children Treated for Malaria in Uganda.

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Background: The WHO recommends the use of ACTs as the first line treatment for malaria. ACTs are highly efficacious, but may differ in their post-treatment prophylactic effect. Few studies have evaluated how nutritional status affects the post-treatment prophylactic effect of ACTs.
Methods: To examine the effect of stunting, an indicator of chronic malnutrition, on the post-treatment prophylactic effect of dihydroartemisin-piperaquine (DP), we prospectively followed 134 children diagnosed with malaria between the ages of 4 to 25 months. All subjects were participants in a cohort study conducted in Uganda, where children were treated repeatedly for malaria over 2 years. Anthropomorphic measurements and malaria outcome data were available for 583 DP treatments dosed according to weight-based guidelines. Height-for-age z-scores (HAZ) were calculated using the 2006 WHO child growth reference standards and stunting was defined as a HAZ value of <-2. The outcome was risk of new malaria infection after 63 days of follow-up excluding recrudescence and patients with unsuccessful genotyping results.
Results: 264 of 583 patients (45%) were classified as stunted at the time they were treated with DP. The mean number of malaria episodes treated during the course of follow up was 3.7 (max=12). Stunted children were more likely to be male, older, HIV-positive, live in a rural area, and were less likely to be taking cotrimoxazole prophylaxis. Using Cox regression with adjustment for repeated measures, stunting was independently associated with a higher risk of new malaria infection after adjustment for age, place of residence, total piperaquine dose, and cotrimoxazole use (best fitting model) (hazard ratio=1.4, p=0.008).
Conclusion: These results suggest that stunting reduces the post-treatment prophylactic effect of DP in children treated for malaria. This may be due to a difference in the pharmacokinetics of DP in stunted children. Pharmacokinetic data from this population is currently being evaluated.

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A capsid-modified adenoviral vector improves immunogenicity as a malaria vaccine

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A variety of vaccine platforms have been tested to develop circumsporozoite (CS) antigen-based malaria vaccine. Although it is well-known that both humoral response and Cell-Mediated Immune responses (CMI) to the CS antigen of rodent malaria parasites are protective against pre-erythrocytic malaria, most vaccine platforms can induce only a biased immune response to either arm; Virus-Like-Particle (VLP) induces immune response biased to humoral and adenovirus (Ad) vector induces response biased to CD8+ T cell. Therefore, a malaria vaccine, which can induce substantially protective humoral response and CMI, has been sought to enhance the efficacy of CS antigen-based vaccine.
In this study, we constructed a novel Ad-based malaria vaccine by inserting a B epitope, $(QGPGAP)_3$, of *Plasmodium yoelii* CS (PyCS) protein into Ad capsid proteins (hexon and/or fiber) to enhance humoral immune response to the PyCS antigen. These capsid-modified Ads showed the same infectivity to AD293 and mouse DC cells as wt CS/Ad *in vitro* and induced high level of PyCS antigen-specific CD8+ T cell response after a single immunization in mice. We also found that after multiple doses of vaccination, the capsid-modified Ads not only augmented PyCS antigen-specific humoral response, but also induced a significant level of protection against malaria challenge. Parasite burden in liver was inversely correlated with anti-QGPGAP antibody titer and sera from mice immunized with capsid-modified Ads strongly neutralized sporozoite infection *in vitro*, which indicated capsid-modified Ads induced protective humoral immune response to the PyCS antigen.
Overall, this study has demonstrated the supremacy of capsid-modifed Ad as a malaria vaccine platform over conventional Ad-based malaria vaccines.

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N-Methylpyrazoles: A Novel Chemical Scaffold Against *Plasmodium falciparum*

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The growing resistance to current first-line antimalarial drugs emphasizes the needs of innovative new chemical classes for the development pipeline. Such chemicals are expected to show activity against multi-drug resistant strains and to hit novel targets to maximize the effect of combination treatment. *N*-methylaminopyrazole we describe here has a novel chemical scaffold as antimalarial drug and was initially identified as a hit scaffold from our robust high-throughput cell-based screen (1,536-well format) based on blood stage proliferation of *Plasmodium falciparum*. The most potent hit compound of the scaffold exhibits good EC_{50}'s of around 500 nM against 15 resistance strains (3D7, W2, HB3, Dd2, etc) and shows low hERG inhibition, CYP inhibition and mammalian cell cytotoxicity. Based on the structure of the initial hit, we performed intensive analog synthesis (ca 300 analogs) and developed structure-activity relationships, focusing on optimization of cellular potency and improvement of ADME and pharmacokinetic properties. A considerable number of the optimized compounds reached to single digit nM cellular potency, and some showed preferable pharmacokinetic properties. Details of the biological profile, *in vitro* ADME, and pharmacokinetic properties of selected compounds will be presented.

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EPIDEMIC DYNAMICS REVEALED IN DENGUE EVOLUTION

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Dengue virus (DENV) is an emerging pathogen typically maintained in endemic transmission in large urban centers of the tropics with periodic epidemics every 3 to 5 years. Puerto Rico (PR), a major population center in the Caribbean, has experienced increasingly severe epidemics since multiple DENV serotypes were introduced beginning in the late 1970s. We document the phylodynamics of DENV-4 between 1981 and 1998, a period of dramatic ecological expansion during which evolution also occurs. The timescale of viral evolution is sufficiently short that viral transmission dynamics can be elucidated from genetic diversity. Virus sequences were acquired by RT-PCR and Sanger sequencing of overlapping amplicons for structural and nonstructural genes from isolates collected throughout PR, dated by month and year. We then applied a coalescent-based approach to estimate virus effective population sizes from sequence diversity of the temporally sampled viruses using Bayesian Markov Chain Monte Carlo methods. Finally, we directly compared our sequence-based estimates of viral effective population sizes through time with the epidemiologic pattern of cyclic outbreaks derived from confirmed case counts. The periodicity of epidemics is strongly correlated with coalescent estimates of effective population size from temporally sampled virus sequences. Thus we confirm that the observed epidemiologic dynamics are associated with periodic severe reductions in genetic diversity compatible with population bottlenecks. Effective population sizes appear to increase prior to isolation counts and then decrease during the intense phase of epidemics, suggesting that strong selection may be acting on fast-growing strains to homogenize and thus reduce genetic diversity after an initial burst. Here we integrate epidemiologic and sequence data in a joint model that demonstrates the significance of genetic bottlenecks and subsequent selection in the evolutionary dynamics of emergent dengue, in an analytical framework that could be used to further explore transmission models of infectious diseases.

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The Use of *Antifungal* Drugs in Culturing *Leishmania* Parasites

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Leishmaniasis is a disease complex caused by 42+ species of protozoan parasites belonging to the genus *Leishmania.*Culturing *Leishmania* parasites for the purpose of the scientific research or clinical diagnosis requires several techniques to ensure proper proliferation of promastigotes.Skin biopsies from potential leishmaniasis patients serve as hosts to many of microorganisms including bacteria, parasites, fungi, and others.Antibacterial drugs such as gentamicin and vancomycin are used to prevent bacterial contaminations in the *Leishmania* cultures.Antibacterial drugs are successful at killing bacteria, however, they do not necessarily eliminate fungal growth.As many of the patient samples are non-sterile sites (like skin) patient cultures are sometimes contaminated with dermal fungi such as the yeast *Candia* species and other fungi including *Cladosporium sp*. and *Aspergillus sp.*These fungi often

interfere with the diagnosis of leishmaniasis and especially with the speciation of *Leishmania.*Identification of the best antifungal candidates would be of great benefit for the diagnosis of leishmaniasis in patient samples and culturing *Leishmania* parasites for research purposes. Since each species of *Leishmania* has different levels of sensitivity to drugs, in this preliminary study, *Leishmania major* and *C. albicans* were selected and tested against ten different anti-fungal drugs.Of the ten drugs applied, four of the drugs showed no effects on *L. major* while actively targeting *C. albicans* and the rest of the drugs either selectively targeted parasites or both parasites and the fungus. *L. major* was extremely resistant and had no significant effects on the proliferation of promastigotes to Amphotericin B, griseofulvin, ketoconazole, and nystatin anti-fungal drugs at higher concentrations. In contrast, L. major was extremely susceptible to clotrimazole, cycloheximide, fluconazole, itraconazole, miconazole nitrate salt, and sulconazole manifested as complete growth arrest when induced by incubation of parasites with higher concentrations of the these anti-fungal drugs for 96 hrs; whereas no changes were observed after 72 hrs. The Leishmania Diagnostic Laboratory is the only College of American Pathology Laboratory certified for Leishmania diagnosis and it is very important to find, discover, or develop new technologies that benefit the patients and help their healthcare physicians with their treatments.

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Optimization of a Primary Drug Screen for Cutaneous Leishmaniasis: Importance of the Length of the Discovery Cycle and Parasite Strain Used

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The infectivity of the mammalian host by the intracellular parasite *Leishmania spp*. varies by species and within a species by strain (isolate). In a drug development program the shorter the discovery cycle, the higher the possibility that a new lead will be discovered. To reduce the discovery cycle, five strains of five different species of Leishmania known to cause cutaneous leishmaniasis (CL) in humans, *L. amazonensis*, *L. guyanensis*, *L. peruviana*, *L. braziliensis*, and *L.tropica*, were selected. These strains were retrieved from the WRAIR Leishmania strains reference bank and their rate of infectivity in rodents was determined. Three Balb/c mice were infected with 10^5, 10^6, and 10^7 *Leishmania* metacyclic promastigotes. Metacyclic promastigotes were obtained by the Ficoll gradient technique. Parasites were thawed and maintained in Schneider's Drosophila Medium plus 20% Fetal Bovine Serum (heat inactivated at 56°C) at 25°C. As in bacteria, Leishmania parasites in culture have three phases: log, metacyclic, and stationary. It was found that the metacyclic phase was the most infective. Metacyclic parasites were defined as six day old cultures with flat parasite counts between day four and day six. A hemacytometer was used to determine the number of parasites per ml. It was found that only parasites that stopped dividing reach their peak infectivity level. To determine the strain with the shortest cycle (infection to ulcer) susceptible Balb/c mice were infected on the base of the tail. Infected animals were observed three times a week to ensure that their environment was clean and they were shaved, so as to allow for infection to occur. As expected, infections begin with a swelling, followed by a papule, and finally a lesion. The first strain to produce a lesion that reaches the ideal size (30 to 50 square millimeters) and developed in the shortest amount of time, preferably within a month, was the strain selected; see Table 1 for results.
Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the army or the Department of defense.

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A novel CD1d-binding NKT cell ligand as a malaria/HIV vaccine adjuvant

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α-galactosylceramide (α-GalCer) has been shown to bind CD1d molecules and activate invariant Natural Killer T (*i*NKT) cells. We have previously shown that α-GalCer can enhance protective CD8+ T-cell responses elicited by various malaria vaccines and increase the level and duration of protection after vaccination. We have also found that α-GalCer enhances the immunogenicity of HIV-1 DNA vaccines in mice, leading to a significant increase in specific T-cell responses and a boost of antibody titers. However, because α-GalCer triggers the secretion of both Th1 and Th2 cytokines from *i*NKT cells, the opposing effects could hamper induction of the optimal adjuvant effect. In this study, we performed step-wise screening assays of a focused library of twenty-five α-GalCer analogs in order to identify a novel glycolipid which provides an adjuvant effect superior to α-GalCer. Assays included quantification of the magnitude and Th1/Th2 cytokine profile after stimulation of *i*NKT cells *in vitro* and *in vivo*, binding affinity to human and murine CD1d molecules, and binding affinity to the invariant TCR (*inv*TCR) of human *i*NKT cells. Through this rigorous screening process, we have identified a lead candidate glycolipid, 7DW8-5, which exhibits a potent adjuvant effect on the humoral and cellular immunogenicity of both malaria and HIV vaccines in mice, and augments protection induced by a recombinant adenovirus expressing *Plasmodium yoelii* circumsporozoite protein against malaria challenge in the rodent model. We are currently pursuing the pre-clinical testing of this compound in animals in order to move forward to a Phase I clinical trial.

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Stability of Alphavirus Attenuation Approaches

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The greatest risk from live-attenuated vaccines is the potential for reversion to virulence. Particular concerns arise for RNA viruses as their polymerase infidelity results in genetic instability that can fuel rapid evolution. While this evolutionary strategy is ideal for virus adaptation and fitness in nature, it poses challenges for the development of safe vaccine candidates. As new technologies emerge and safety requirements become more stringent, it is important to incorporate lessons learned from existing vaccines and apply them to current attenuation strategies in development. We examined the genetic stability of 3 live-attenuated vaccine strategies for the alphavirus, Venezuelan equine encephalitis virus (VEEV). We compared the only currently utilized human VEE vaccine, TC-83, which was generated through 83 serial passages through guinea pig hearts, to a rationally designed vaccine candidate: strain V3526, with two designer mutations, and to a chimeric alphavirus generated from a Sindbis virus backbone with VEEV-derived structural protein genes (SIN/TC/ZPC). Each virus, recovered from a cDNA clone, was serially passaged 10 times through intracranial inoculations in infant mice to select for reversion to neurovirulence. Each strain was evaluated for changes in virulence at passages 5 and 10 by inoculating suckling mice subcutaneously and measuring survival. We also compared the genomic sequences of the parental viruses to those of the passaged strains. All three of the vaccine strains exhibited some degree of instability, with increases in virulence. Of the 3 vaccine strains, TC-83 and SIN/TC/ZPC were the most attenuated initially. However, V3526 and SIN/TC/ZPC exhibited the least virulence increases. By passage 10, SIN/TC/ZPC caused only 44% mortality, whereas the other vaccine candidates killed all mice. Sequence analyses indicated that the SIN/TC/ZPC strain exhibited the greatest genetic stability, with only one amino acid change occurring in the nonstructural protein 3 (nsP3). TC-83 acquired 3 nonsymonymous mutations in the nsP2, E2 and E1 genes, while V3526 underwent 3 nonsymonymous substitutions in the E2 and E1 genes. Our findings suggest that the chimeric SIN/TC/ZPC strain combines the initial attenuation of TC-83 with the phenotypic stability of V3526 and highlight the importance of the stability of attenuation strategies for maximizing vaccine safety.
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Identification of Putative Molecular Determinants of Dengue Virus Serotype 2 Strains Conferring Resistance to Interferon Signaling

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The acute nature of dengue virus (DENV) infections suggests that the innate immune system plays a vital role in its elimination. Gene expression profiles show that the type I interferon system (IFN-α/β) is highly up-regulated in patients with dengue fever, whereas there is suppression of IFN-α/β-stimulated genes in cases of dengue hemorrhagic fever or shock syndrome. Moreover, DENV suppresses IFN-α/β signaling in infected cells through the action of two viral nonstructural proteins. NS4B suppresses STAT1 phosphorylation and NS5 triggers STAT2 degradation. These observations suggest that these mechanisms of DENV moderate the antiviral response, possibly affecting disease outcome. In order to determine if DENV strains differ in their ability to block IFN-α/β signaling, and identify natural molecular determinants of IFN resistance, we examined several DENV-2 American, Asian, American/Asian, Cosmopolitan, and Sylvatic strains for their ability to block IFN-α/β signaling. Replication assays showed that these strains display varying degrees of sensitivity to IFN-α/β. To further characterize the ability of these viruses to modulate IFN-α/β signaling, we examined expression and phosphorylation of STAT1 and STAT2 in infected VERO cells after IFN-α/β stimulation. Our results showed that these viruses varied in their ability to block IFN-α/β signaling through inhibition of STAT1 phosphorylation. We cloned the NS4B gene from the DENV-2 strains studied into a mammalian expression vector to confirm our previous results and are currently performing site-directed mutagenesis of NS4B coding sequences to validate these observations. While NS4B function varies among virus strains, NS5 mediated degradation of STAT2 was homogeneously observed in all of the virus strains examined, demonstrating that these viruses possess at least one mechanism for blocking IFN-α/β signaling. The observed variable IFN-α/β signaling antagonism among DENV-2 strains suggests that this mechanism may be a key element among the plethora of factors that determine clinical outcomes resulting from infections.

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Worms Come to Town: Screening for Subclinical Parasitic Infections among Foreign-born Patients in an Urban HIV-care Clinic in Atlanta, GA

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Background: In some areas, an increasing number of HIV diagnoses are in foreign-born persons at risk for latent untreated parasitic infections. The long-term consequences of such co-infections can be severe, yet little data exist on parasite prevalence in this population.
Methods: This cross-sectional study evaluated 100 foreign-born persons at one HIV clinic in 2009. We determined the prevalence of infection with strongyloidiasis, schistosomiasis, lymphatic filariasis, cysticercosis and Chagas disease using serologic testing. We also studied associations between helminthic infection and peripheral eosinophilia, CD4 count and symptoms based on chart reviews and questionnaires.
Results: Of the 100 participants, 72 were male and the median age was 40 years (range 21-64); 60 were from Mexico and Latin America, 25 from Africa, and the remaining 15 from Asia and the Caribbean. *Strongyloides stercoralis* enzyme immunoassay testing was positive in 25/100 (25%). Of the 10/36 (28%) with positive schistosomiasis serologic testing, 4/10 (40%) had antibodies to *S. mansoni*, 4/10 (40%) to *S. haematobium*, and 2/10 (20%) to both species; 9/10 (90%) of participants did not recall prior schistosomiasis treatment. None tested positive for Chagas disease, lymphatic filariasis or cysticercosis. The median absolute eosinophil count was 128 cells/mm^3 (range 0-1476) and median CD4 count was 297 cells/mm^3 (range 0-1757); neither eosinophilia nor CD4 count was associated with strongyloidiasis or schistosomiasis. Abdominal pain was present among 8/25 (32%) and 2/10 (20%) with strongyloidiasis and schistosomiasis respectively.
Conclusion: Given the high prevalence rates of certain helminths and the potential lack of eosinophilia and suggestive symptoms, selected screening based on risk history may be appropriate among foreign-born HIV-infected patients. Identifying and treating helminth infections could help prevent long-term complications in this vulnerable population.

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Recovery of intestinal parasites from naturally contaminated hands of children living in low socio-economic areas of Dhaka, Bangladesh

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Intestinal parasitic infections are global problem with more than an estimated one billion infected persons mostly in underdeveloped countries. Children are most effected by these infections. This is predominantly due to poor hygienic conditions, low parenteral health education, and absence of safe drinking water. The scientific evidence describing intestinal parasite and bacterial contamination on paper currency from a developing country highlight the role of poor hand hygiene practices promoting dissemination of infectious diseases including intestinal parasitic egg / (oo)cysts. As a part of parasitological survey to assess the intestinal parasites amongst low socio-economic communities of Indian sub-continent stool and hands were analyzed for evidence of parasite eggs / (oo)cysts from children living in slums of Dhaka, Bangladesh. A total of 215 stool samples have been analyzed for intestinal parasitic eggs / (oo)cysts using conventional microscopic assay. The general prevalence in this part of the survey of intestinal parasitic egg / (oo)cysts were 37% (79 positive out of 215 stool samples). Of these 79 stool-positive children, to date hands of 65 were sampled in order to screen for any intestinal parasitic egg / (oo)cyst. Three different types of parasitic egg / cysts were recovered from hands of these children. Amongst the helminths, Ascaris 11%, and Trichuris 1.5% were recovered where as the protozoan parasite, Giardia recovery was 5% of hand samples. Over all, ≥ 17% of the children examined carried intestinal parasitic egg / cysts on their hands and 37% in their stool samples, indicating the perpetuation of fecal-hand-mouth cycle of helminths and protozoan infections in these children. These samples are currently being further characterized by cellulalr and molecular methodologies. To our knowledge this the first report where intestinal parasites have been recovered from naturally-contaminated hand samples of children. Proper hand hygiene plays a paramount role to effectively disrupt the chain of fecal-oral transmission of infectious diseases including the intestinal parasites. This highlight the need to include proper hand hygiene practices (washing hands with soap and water) including health hygiene educational promotion programs in order to sustain any chemotherapeutic programs. The data being generated in this survey will be presented and its implications on public health will be discussed.

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Use of oral cholera vaccines in an outbreak in Vietnam, implications for cholera control

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Objective: The role of killed oral cholera vaccines (OCV) in controlling outbreaks needs to be assessed. OCVs are available but not used routinely for cholera control except in Vietnam. In 2007 and 2008, Vietnam's capital, Hanoi, experienced unprecedented cholera outbreaks and a decision was made to immunize two districts. Here we assess the impact of OCV once a cholera outbreak has begun.
Methods: From 16 to 28 January 2008, mass vaccination campaigns were held in two districts of Hanoi. No cholera cases were detected from 5 February to 4 March 2008, after which cases were again identified. Beginning 8 April 2008, residents of two vaccinated and two similar unvaccinated districts of Hanoi admitted to one of five hospitals for acute diarrheal illness with onset after 5 March 2008 were recruited for a matched, hospital-based, case-control study. Subjects were matched by hospital admission date, district, gender and age to controls admitted for non-infectious conditions. In adjusting for potential confounders, all variables that differed statistically significantly among cases and controls were fitted together with vaccination status. Odds ratios with 95% CI were calculated by multivariate conditional logistic regression and vaccine effectiveness calculated as (1- OR) x 100.
Findings: We were able to include 126 diarrheal cases and 126 controls hospitalized for other medical conditions matched on admission date, age, gender and district of residence. These were less than the required sample size. Of subjects residing in the area where the mass vaccination campaign was carried out, 22 of 108 subjects (20%) received cholera vaccine. No subjects from the unvaccinated districts received cholera vaccine. The vaccine was 77% protective against cholera (OR 0.23; 95% CI 0.06 to 0.92, *P*=0.038) after adjusting for intake of dog meat or raw vegetables and drinking bottled or boiled water most of the time.
Conclusion: This is the first study to explore the use of the killed OCV during a cholera outbreak. Our findings suggest that killed OCVs may have a role in controlling cholera outbreaks. Further evaluation is warranted.

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Allelic diversity of the sex peptide receptor homolog in Anopheles gambiae from the Kilombero district of Tanzania

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Little is known regarding the impact of mating on behavior of the female malaria vector *Anopheles gambiae*. Due to the human health impacts associated with *An. gambiae*, it is important to understand aspects of the mating biology, particularly those which can affect disease transmission dynamics. In other insect species dramatic post-mating changes in behavior, such as decreased female receptivity, increased egg production and egg laying, and increased feeding behavior, have been observed. These behavioral changes are due to seminal fluid proteins (SFPs) which are transferred from the male to the female during mating. Sex peptide is a major SFP which may cause several female post-mating changes. Recently, the sex peptide receptor (SPR) for *D. melanogaster* was identified. We have identified a putative SPR homolog in the malaria vector *An. gambiae*. We examined the allelic diversity of the SPR homolog in field-collected female *An. gambiae* from the Kilombero district of Tanzania. Potential implications of our results on population genetics and mating biology will be addressed.

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The evolutionary analysis of Japanese encephalitis virus and the molecular characterization of genotype II of the virus

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Previous investigations suggest that Japanese encephalitis virus (JEV) originated in the Indonesia-Malaysia region from an ancestral virus common to JEV and Murray Valley encephalitis virus. From this ancestral virus GIV of JEV diverged from the most recent common ancestor (MRCA) approximately 350 years before present (YBP), followed by GI-GIII. The more recent genotypes have spread to other areas, while GIV appears to be confined to Indonesia. The time frame by which GI-III of JEV diverged from the MRCA, the rate of evolution of these three genotypes and the genomic molecular characterization of GII viruses remains to be elucidated.
To infer the time frame and rate of JEV evolution, Bayesian analyses were performed on a nucleotide sequence alignment consisting of the open reading frame of 33 JEV isolates, including three newly sequenced GII isolates. The deduced amino acid sequences of the three novel GII isolates were compared to each other, in addition to the only other fully sequenced GII isolate.
Subsequent to the divergence of GIV of JEV (327 YBP), the MRCA of GI+GII+GIII diverged (209 YBP) followed by the MRCA of GI+GII (129 YBP), the MRCA of GIII (123 YBP), the MRCA of GII (79 YBP), and lastly the MRCA of GI (51 YBP). The highest rate of molecular evolution (substitutions/1000 nucleotides/year) was observed in GI (1.06), followed by GIII (0.29) and GII (0.19). The genomic intragenotypic divergence among the four GII isolates ranged from 0.5-0.9%. Interestingly, the most recent GII isolate

possessed six distinct amino acid substitutions within the envelope protein, a protein involved in host cell receptor binding and virus entry, compared to the other three GII isolates.
JEV recently originated from its ancestral virus and evolved into at least four genotypes, three of which have arisen within the last 209 years and appear to have distinct evolutionary rates which may be associated with their epidemiological histories. The study of the evolution of JEV will provide clues to how this virus and other flaviviruses are successfully expanding into new global habits.

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Emergence of a Novel Human Ehrlichia Agent in the Midwestern United States, 2009

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Introduction: We report the preliminary characterization of a novel *Ehrlichia* agent detected in association with febrile illness in 3 Midwestern U.S. patients and provide corresponding clinical and epidemiological data.
Methods: DNA was extracted from EDTA whole blood of acutely ill patients and tested for *Ehrlichia* and *Anaplasma* sp. using *gro*EL real-time PCR and melting temperature (Tm) analysis. Positive specimens were further characterized by sequencing fragments of *gro*EL and 16S rRNA (*rrs*) genes and culture isolation. Patients were interviewed by Local and State Health Departments to obtain demographic, clinical and epidemiologic information.
Results: In summer 2009, DNA from two febrile Wisconsin men and one Minnesota woman were *gro*EL PCR positive with an atypical Tm, distinct from that of *Ehrlichia chaffeensis*, *E. ewingii* and *Anaplasma phagocytophilum*. All were also positive by SYBR-Green PCR targeting *rrs* of *Anaplasmataceae.* A sustained *Ehrlichia* isolate (designated Wisconsin) was cultured from the blood of one patient. The *rrs* nucleotide sequences from 3 clinical specimens and the culture isolate were identical to each other and most similar to homologous *rrs* fragments of *E. muris* and uncultivated *Ehrlichia* sp. found in *Ixodes ovatus* and *I. persulcatus* in Japan. All three patients reported fever and headache; one had a rash. Lymphopenia and thrombocytopenia were present in all 3 patients, and mild elevation of hepatic transaminases in one. One patient, a bilateral lung transplant recipient, was hospitalized for 3 days. All patients were treated with doxycycline and recovered. Factors associated with illness included exposure to wooded areas and ticks.
Conclusions: A novel *Ehrlichia* agent pathogenic for humans is described from a confined geographic region in the Midwestern U.S. Further assessment of its ecology, epidemiology and the spectrum of illness it causes are important to facilitate its distinction from other vector-borne infections known in this region and elsewhere.

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A case on distributing survey of snail-borne disease by combining GIS technique with field investigate

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Angiostrongyliasis cantonensis which is belonging to one of snail-borne parasitic diseases is an emerging infectious disease in the mainland of China. Several important public health events have been records due to the disease caused by *Angiostrongylus*. One is biggest event was took place in Beijing located in the north of China in 2006, and involved 160 infected individuals. Normally angiostrongliasis can be found in the southeastern coastal regions. The questions are 1) whether angiostrongyliasis had changed its endemic areas 2) what is its distribution characteristic in the mainland of China.
After reviewed the outbreaks records, it found most of the events was due to eat the snails, *Pomacea canaliculata* . *P.canaliculata* is the main intermediate of *A. cantonensis* in the mainland of China. The potential range of *A. cantonensis* and its main intermediate host were predicted based on the Degree-Day models using GIS technique. The grid sampling was performed on the prediction map and 5% grids were random sampled. 55 random samples were selected. The survey on *A. cantonensis* and its hosts was conducted in one specific village in each grid in the same time, during Sep-Oct, 2006. Totally 19 provinces in the mainland of China were predicted as potential habitats of *Pomacea canaliculata*. As a result,the snails were distributed in Fujian, Jiangxi, Zhejiang, Hunan, Guangdong, Guangxi, Hainan and Yunnan province. All of the provinces but Yunnan were identified as the natural epidemic focuses in this survey. Meanwhile,the highest natural prevalences of *P. canaliculata* in each province were 36.62% in Jianou Fujian, and 19.87% in Xingguo Jiangxi, 16.00% in Ruian Zhejiang, 5.00% in Rucheng Hunan, 6.31% in Huazhou Guangdong, 39.09% in Shangsi Guangxi and 25.00% in Wuzhishan Hainan, respectively. Also, Yunnan is found one of the natural epidemic focuses after taking farther survey in 2009.
That is a feasible way to confirm the endemic areas and discovery of natural epidemic foci of *A. cantoennsis*, a kind of snail-borne disease, using combination of GIS technique and field survey.

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Cytochrome P450 Reaction Phenotyping of a Novel Anti-Malarial Drug Candidate

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The Cytochrome P450 (CYP) superfamily of enzymes is an extremely large and diverse set of heme containing enzymes found across all kingdoms of life. These enzymes play a pivotal role in the metabolism of xenobiotics in the human liver. Studying the metabolism and disposition of a potential drug candidate is a crucial part of the drug discovery/development process as potential drug-drug interactions as well as the nature of putative metabolites should be well documented to guide lead optimization. In order to identify the number and type of enzymes involved in the metabolism of a novel anti-malarial drug candidate, WR319538, the compound was incubated with the five most abundant CYP enzymes found in the human liver and flavin containing monooxygenase 3 (FMO3),. Parent compound concentration was monitored at several time-points throughout the incubation using LC-MS. The kinetics of metabolically active enzymes against WR319538 were characterized. The contribution of each enzyme was ordered by relative abundance weighted intrinsic clearance (Vmax/Km). Experiments indicate that CYP 3A4 plays the most dominant role in WR319538 metabolism, with an abundance weighted intrinsic clearance of 1.6 s^{-1}, followed by CYP 2C9 (0.83 s^{-1}) and 2D6 (0.16 s^{-1}) respectively.

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INTERFERENCE OF DEXAMETHASONE IN THE PULMONARY CYCLE OF *STRONGYLOIDES VENEZUELENSIS*

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Introduction and Objectives *Strongyloides* spp are nematode parasites with an obligatory pulmonary phase of the life cycle, typically occurring within hours after infection and lasting only a few days before the worms migrate to the intestine, where they develop into adults. Little is known about the consequences of the passage of larvae in the pulmonary capillaries in nematode infections. Activated Th2 lymphocytes orchestrate the inflammatory cascade, communicating with the two primary effector cells, the mast cell and the eosinophil, by release of interleukins 4 (IL-4) and 5 (IL-5). The objective of this study was to investigate the interference of a daily treatment of dexamethasone in the immunomodulation of pulmonary response of *Strongyloides venezuelensis* infection in rats.
Methods and Results Animals were divided into four groups: (C) Control group; (CD) Control and Dexamethasone group; (I) Infected group; (ID) Infected and Dexamethasone group. The groups (I) and (ID) were inoculated with 9,000 *S. venezuelensis* infective larvae L3. The (CD) and (ID) groups received 2 mg/kg daily of dexamethasone. At 1, 3, 5, 7, 14 and 21 days post-infection, the animals were killed. Three principal effects were found associated to dexamethasone treatment: 1) Increased alveolar hemorrhagic inflammation provoked by the passage of larvae into alveolar spaces; 2) Significant decrease of eosinophil and mast cell migration to the axial septum of the lungs and 3) Impaired formation of the reticular fiber network, interfering with granuloma organization.
Conclusion This paper showed that the use of drugs with immunomodulatory actions, such as dexamethasone, in addition to interfering with the morbidity from the pulmonary cycle of *S. venezuelensis* infection, may contribute to revealing the mechanisms involved in its pathogenesis.*This study was published on Am J Trop Med Hyg 2008; 79(4):571-578*

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Dexamethasone therapy induces *Strongyloides venezuelensis* hyperinfection in rats through immunomodulation cellular and increased ecdyse-like hormone expression in the parasites

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Introduction *Strongyloides* spp are nematode parasites that has a complex life cycle, with the larvae penetrating the skin and travelling through the bloodstream to the lungs, and continue their development in the gut. Autoinfection has been described as a peculiar aspect of this parasitosis, however, in immunocompromised hosts, an accelerated infective cycle can result in a hyperinfection, potentially leading to multi-organ failure and death. To gain insight into the mechanisms underlying dexamethasone-induced alterations in the cycle of *Strongyloides venezuelensis* (Sv), we infected rats, a natural host of Sv, and treated them with dexamethasone.
Methods and Results Rats were divided into three groups: Control group; Sv+PBS group, and Sv+Dexa group. The Sv groups were inoculated with 9,000 Sv larvae. The Sv+Dexa group received 2 mg/kg of dexamethasone. At 1, 3, 5, 7, 14 and 21 days, the animals were killed. Inflammatory cells were quantified in lung tissue, blood, peritoneal cavity fluid (PCF) and bronchoalveolar lavage fluid

(BALF). Eosinophil peroxidase (EPO) was measured in PCF and BALF. Corticosterone, ecdysterone were measured by radioimmunoassay in larvae and female. Glucocorticoids receptors were quantified in extract of worms by Western Blotting. The infection promoted an increase of eosinophil and mononuclear cells numbers in all compartments, with high EPO production. Dexa treatment inhibited inflammatory cells in blood, PCF and BALF, lung and mucosa tissue. Moreover, dexa treatment improved parasite fertility and proliferation, probably throughout increased synthesis of corticosterone and hormones ecdysterone-like in worms, and inducing glucocorticoids receptors expression. Importantly, dissemination of larvae to lungs, spleen, kidneys, heart, liver, and brain were seen, confirming hyperinfection.
Conclusions These data confirm that dexamethasone therapy induces *Strongyloides venezuelensis* hyperinfection in rats through immunomodulation cellular and increased ecdyse-like hormone expression in the parasites resulting in higher fertility and dissemination.

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A genome-wide scan for novel drug resistance loci in Plasmodium falciparum

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Plasmodium falciparum exhibits substantial genetic diversity, which allows this human pathogen to both escape host immunity and develop drug resistance. Understanding this genetic diversity is critical to the development of effective therapeutic and intervention strategies. We have begun conducting genome-wide association studies (GWAS) in the malaria parasite using a malaria SNP genotyping array and a high-throughput platform for the characterization of drug phenotypes. Even with a limited number of samples, using this approach we were able to identify with genome-wide significance two well-known drug resistance loci, containing the *pfcrt* and *dhfr* genes, as well as an additional novel locus on chromosome 10 (Pf10_0355) associated with resistance to halofantrine. Transgenic parasites that overexpress a "resistant" copy of Pf10_0355 display decreased sensitivity to both halofantrine and mefloquine. Additionally, we find copy number variation at the Pf10_0355 locus, which appears to contribute to the decreased drug susceptibility that we observe. Our results demonstrate the power of genome-wide scans to identify novel loci contributing to malaria drug resistance and point to a role for Pf10_0355 in modulating halofantrine and mefloquine resistance in *Plasmodium falciparum.*

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A possible role of inflammatory cytokines and proteases activation in the degradation of dystrophin in hearts of mice experimentally-infected with *Trypanosoma cruzi.*

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Background. Previous studies from our laboratory demonstrated the coincidence between peaks of inflammation and mortality and reduced expression of dystrophin. This study was carried out to evaluate the role of inflammatory cytokines and the activation of proteases in the degradation of dystrophin in hearts of mice experimentally-infected with *Trypanosoma cruzi*.
Methods. C57Bl/6 mice were infected with Y strain of *Trypanosoma cruzi*. The animals were killed 9, 14, 20, 26 and 32 days after infection and parasitemia and mortality rate were observed. Immunofluorescence staining and Western blotting were performed for TNF-alfa, iNOS, and calpain. Sarcolemmal permeability was evaluated by albumin staining.
Results. Mice displayed a parasitemic peak at Day 9 while the mortality rate reached a peak on Day 20. Lymphomononuclear inflammatory infiltrate was present at Day 14, peaking at Day 20 with pronounced intensity that became less intense at Days 26 and 32. A progressively increased expression of TNF-alpha, iNOS, and calpain was observed on the immunoflorescence and Western blotting analysis peaking at Day 20 post-infection. The sarcolemmal permeability analysis demonstrated that albumin was localized in the interstitial space as a delicate network and in the vascular lumina in controls animals. In infected mice, mainly on Day 20, albumin staining was more intense in the interstitial space and spread blocks of myocytes became positives.
Conclusions. Loss of cell membrane integrity may result in Ca^{2+} overload to cardiomyocytes, and cytokines secreted by inflammatory cells, such as TNF-alpha and reactive oxygen intermediates, could activate nuclear factor-κB and contribute to the progression of the dystrophic damage through activation of intracellular proteases, mainly calpain.

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Lifecycle establishment and morphological observations of *Armillifer agkistrodontis* (Pentastomida), a new zoonotic parasite from China

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Pentastomiasis is a rare parasitic infection in humans and few data regarding the biology and morphology of Pentastomida are currently available. In this study, the lifecycle of *Armillifer agkistrodontis* was established in mice and rats as intermediate hosts, and in snakes (*Agkistrodon acutus* and *Python molurus*) as end hosts under laboratory conditions. Different stages of the parasite including eggs, larvae and adults were isolated and examined morphologically using light and electron microscopes. Both mice and rats were easily infected, and the time for complementation of the *A. agkistrodontis* lifecycle was about 14 months including 4 months for the development from the egg to the infectious larva in the intermediate host and 10 months from the infectious larva into a mature adult in final host. The main morphological difference between *A. armillatus* and *L. serrata* is the number of abdominal annuli; 7-9 in *A. agkistrodontis* and around 80 in *L. serrata*.

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Risk factors associated with the presence of *Aedes aegypti* pupae in San Martin de Porras, Lima, Peru

Introduction
Aedes (Stegomya) aegypti is the primary vector of dengue and urban-acquired yellow fever in subtropical and tropical areas of the Americas. A. aegypti has reemerged in Lima since 2000, accompanied by dengue since 2005. The main purpose of this study was to determine factors which allowed *A. aegypti* pupae to reestablish in one district of Lima.
Material and Methods
A cross-sectional study was conducted, sampling randomly 16 localities of San Martin de Porras district, Lima, Peru. The following *A. aegypti* pupae indices were calculated: 1) pupae house index (PHI)--the percentage of housing units with pupae-positive containers; 2) the pupae Breteau index (PBI)--the number of pupae-positive containers per 100 housing units; 3) the 'pupae-per-person' index (PPPI)--the number of pupae per inhabitant; and 4) the pupae density (PD)--the number of pupae per housing unit. Using a negative binomial regression we modeled the number of pupae with the following variables: housing materials; water supply and storage systems; sewage system; type, size, and location of water container with pupae; exposure of water container to sun and light.

Results
In total 7,110 houses (approximately 10% of the total houses), 36,234 inhabitants and 8,540 containers were assessed. We found a total of 1,581 *A. aegypti* pupae in a total of 206 houses and 229 containers. Estimated *A. aegypti* pupae indices were: PHI, 2.9% (95% confidence interval [CI]: 2.5-3.3); PBI, 3.2% (95% CI: 2.8-3.7), a PPPI of 4.4% (95% CI: 4.2-4.6) and a PD of 0.22 (95% CI: 0.21-0.23). The majority of pupae were recovered either from flower containers (606; 38.3%) or plastic containers (600; 38.0%). There were significantly higher levels of pupae in containers which were not refilled within seven days (rate ratio=8.3, 95%IC: 3.6-18.9) and in the flower containers (rate ratio=2.57, 95%IC: 1.5-4.4).
Discussion
These results suggest that in areas with low *A. aegypti* pupae indices such San Martin de Porras, the presence of *A. aegypti* pupae is associated with delayed refilling (≥7 days) of water in containers and with the use of flower containers.

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Risk factors associated with the presence of *Aedes aegypti* pupae in San Martin de Porras, Lima, Peru.

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Aedes aegypti is the primary vector of dengue and urban-acquired yellow fever in subtropical and tropical areas of the Americas. *Ae. aegypti* resurfaced in Lima in 2000 resulting in cases of dengue since 2005. The main purpose of this study was to determine the main factors that allowed the reestablishment of *Ae. aegypti* in San Martin de Porras, Lima Peru.
A cross-sectional study was conducted by randomly sampling 16 sites in San Martin de Porras. In total 7,110 houses (approximately 10% of the total houses), 36,234 inhabitants and 8,540 containers were assessed. The pupae of *Ae. aegypti* were counted in each site and these indices were calculated: 1) pupae house index (PHI)--the percentage of housing units with pupae-positive containers; 2) the pupae Breteau index (PBI)--the number of pupae-positive containers per 100 housing units; 3) the 'pupae-per-person (PPP)--the number of pupae per inhabitant; and 4) the pupae density (PD)--the number of pupae per housing unit. Using a negative binomial regression we modeled the number of pupae with the following variables: housing materials; water supply and storage systems; sewage system; type, size, and location of water containers; exposure of water container to light.

We found a total of 1,581 *Ae. aegypti* pupae in 206 houses within 229 containers. *Ae. aegypti* pupae indices were: PHI, 2.9% (95%

confidence interval [CI]: 2.5-3.3); PBI, 3.2% (95% CI: 2.8-3.7), a PPP of 0.44 (95% CI: 0.42-0.46) and a PD of 0.22 (95% CI: 0.21-0.23). The majority of pupae were recovered from flower containers (606; 38.3%) and plastic containers (600; 38.0%). There were significantly higher pupal counts in containers that were not refilled within seven days (rate ratio=8.3, 95%IC: 3.6-18.9) and in the flower pots (rate ratio=2.57, 95%IC: 1.5-4.4).
These results suggest that the presence of *Ae. aegypti* pupae is closely associated with delayed refilling (≥7 days) of water in containers and with the use of flower pots.

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Using Flow Cytometry to Discover Stage Specific Antimalarial Drugs

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Treating the vast number of patients infected with malaria has resulted in widespread resistance to current antimalarial drugs and a limited number of effective drug therapies. Not only is the current antimalarial drug arsenal in a fragile state, there is also a very limited understanding of how existing antimalarial drugs work against Plasmodium species parasites. However, there are presently intense efforts ongoing to discover more structurally diverse antimalarials beyond the four currently available classes of compounds: antifolates, aminoquinolines, artemisinin derivatives, and the hydroxynaphthoquinone atovaquone. It is anticipated that these efforts will lead to antimalarial drug candidates that should not be primed for the rapid emergence of resistance. It has also become important to develop better methods to evaluate parasite drug susceptibility through in vitro studies. Here, in a small pilot study, we demonstrate the effectiveness of a modified flow cytometry technique for screening small molecule compound libraries for overall antimalarial activity and highlight its usefulness to determine life cycle stage drug susceptibility of Plasmodium falciparum in vitro asynchronous cultures. This study revealed the discovery of three new structurally diverse antimalarial compounds, Bay 43-9006 (an inhibitor of tyrosine receptor and serine-threonine kinases), SU 11274 (a MET kinase inhibitor), and TMC 125 (non-nucleoside reverse transcriptase inhibitor) all of which exhibit potent (<1 μM) overall and ring stage in vitro antimalarial activity with low toxicity to erythrocytes.

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Preclinical and Clinical Testing of a Recombinant Subunit Vaccine for Dengue

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Dengue viruses are a major cause of morbidity and mortality throughout the tropics and subtropics with an estimated 50-100 million cases of dengue annually. To date no specific vaccine or therapy has been developed to combat this important disease. Hawaii Biotech, Inc. is developing a tetravalent recombinant subunit vaccine to protect individuals against dengue virus induced disease. GMP manufacture of the four truncated dengue envelope proteins to support human clinical trials has been completed. Preclinical studies conducted in mice and rabbits have demonstrated the immunogenicity of both monovalent and tetravalent formulations adjuvanted with alum, and formal toxicology studies have demonstrated acceptable safety. An alum-based dengue 1 formulation was shown to be immunogenic and protective in non-human primates. As a precursor to clinical testing of the tetravalent subunit vaccine, a Phase 1 clinical study of monovalent DEN1 is being conducted in healthy volunteers. Preparations for initiation of a Phase 1 clinical study of the tetravalent subunit vaccine have commenced.

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Incidence of Typhoid Bacteremia in Children under 2 years in Karachi, Pakistan

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INTRODUCTION: The burden of typhoid fever in pre-school children is not well recognized. The purpose of this study was to estimate the incidence of typhoid bacteremia in Pakistani children < 5 years of age, and consider the implications of these findings for immunization policies in highly endemic countries.
METHODS: Household surveillance from February 1, 2007 to May 12, 2008, was carried out by community health workers, in 2 low-income, coastal communities of Karachi. Workers referred each sick child < 5 years old to the local clinic. Blood for culture was obtained from those who gave consent, and inoculated in BACTEC Peds Plus® (Becton Dickinson, Sparks, MD, USA) bottles and processed per manufacturer's guidelines.
RESULTS: Overall, 5,570 children contributed 3,949 observation years. Blood culture was obtained from 1,165 cases, yielding 36 pathogens. Salmonella Typhi was isolated in 16 cases, Salmonella Paratyphi A in 2 cases, and Salmonella Paratyphi B in 1 case. The

incidence of typhoid bacteremia in children < 2 years was 443.1 (95% CI: 193.8 - 876.5) per 100,000 child-years. The overall incidence rate of typhoid for children < 5 years was 405.1 (95% CI: 239.8 - 643.9) per 100,000 child-years.
CONCLUSION: Typhoid is a common and significant cause of morbidity among young children in Pakistan, including children less than 2 years of age. Vaccines that provide protection to pre-school children should be included in typhoid control efforts.

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Development of a Recombinant Subunit Vaccine for West Nile Virus

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The first documented case of West Nile virus infection was in the West Nile region of Uganda in 1937. It has since spread through the Middle East, parts of Europe and Asia, and emerged in the Americas a decade ago. Since the first case of human infection in the U.S. in New York City in 1999, the virus rapidly spread throughout the North American continent and is now spreading throughout South America. With annual incidence rates in the U.S. alone generally in the thousands and relatively high incidence of neuroinvasive disease with long term sequelae, development of a safe, effective vaccine that can protect healthy and immunocompromised individuals remains an important goal. Hawaii Biotech Inc. has developed a recombinant subunit vaccine (HBV-002) based on the envelope glycoprotein of West Nile virus formulated with alum. The vaccine has been shown to be safe and effective in several relevant animal models including the golden hamster. In a Phase 1 human safety study, the vaccine was found to be safe and immunogenic. The clinical study of 24 healthy subjects evaluated three different doses of the HBV-002 vaccine and found that all doses of 5, 15 and 50 micrograms of active ingredient were well tolerated. Furthermore all subjects vaccinated with the alum-formulated vaccine developed virus neutralizing antibodies. Clinical study findings will be presented.

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Using Client Exit Interview to Enhance Malaria in Pregnancy Prevention and Control by Antenatal Health Workers in Akwa Ibom State, Nigeria

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Abstract
Background: Malaria in pregnancy (MIP) is a major public health concern in Nigeria. In 2005, Nigeria adopted national guidelines for malaria prevention and control during pregnancy as a component of Antenatal Care (ANC). The strategies included use of insecticide treated nets (ITNs), intermittent preventive treatment of malaria in pregnancy (IPTp) using sulphadoxine-pyrimethamine and malaria treatment seeking with appropriate case management of malaria. This study used client exit interviews to assess the performance of antenatal care frontline health workers in 15 primary health care centers in Akwa Ibom State with feedback provided as a means to improve service delivery.
Methods: 220 clients were drawn from 15 primary health care centers purposively selected from four local governments. Three rounds of interviews were carried out from October 2008 to August 2009. Five pregnant women were selected per facility per round. The interviewing started with the first client exiting the clinic followed by the next client exiting after each interview was complete until five were interviewed. The one-page instrument sought information on services received at the ANC visit and were verified by checking the ANC card. The result of each round of interviews was used to provide feedback to the facility staff to enhance quality of service provision.
Results: Only 16% of respondents (n=220) received ITNs overall, and there was no improvement over time. The provision of any IPTp doses increased slowly over time from 60% to 61% to 67%. Reports of Counseling about IPTp increased from 77% to 100% over the period. Counseling on ITN use showed a similar trend (74%, 80%, 89%). Improvements in providing folic acid and iron supplements to ANC clients also showed marked improvement over time.
Conclusion: Feedback to staff appears to help them focus on improving quality of malaria in pregnancy services in the context of ANC at local health facilities. The biggest challenge to service improvement appears to be the availability of commodities, particularly ITNs. This type of information will be used in advocacy to get local government and state program managers to ensure that ANC staff have the supplies they need to prevent malaria in pregnant women.

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Effect of village-wide use of Long Lasting Impregnated Nets on Leishmania donovani infection in India and Nepal: cluster randomised trial.

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Background: Visceral Leishmaniasis (VL) control in Indian subcontinent is currently based on case detection and treatment and vector control using Indoor Residual Spraying (IRS). Long Lasting Impregnated Nets (LN) has been postulated as an alternative or complement to IRS. We tested the impact of comprehensive provision of LN in VL endemic villages on *Leishmania donovani* infection.
Methods: Cluster randomised controlled trial in 26 VL endemic clusters (16 in India and 10 in Nepal) aiming to detect a 50% reduction in *L. donovani* infection incidence rates in the intervention group compared to control. The secondary endpoint was incidence of VL cases. The number of seroconversions was determined by Direct Agglutination Test (DAT) at 12 and 24 months post-intervention. Seroconversion was only considered in DAT negative (<1:1600) individuals at baseline (December 2006). Assumptions underlying sample size were a 2% yearly *L. donovani* infection incidence; 500 inhabitants per clusters with an anticipated 10% loss to follow up, 20% of the population non eligible and a coefficient of correlation between clusters (k) of 0.25. Under those assumptions a power of 90% with an alpha-risk of 5% was anticipated in a 2 year follow up. In December 2006, LNs (PermaNet® 2.0) were distributed to all households in the intervention clusters. The odds for seroconversion were analysed using a mixed logistic regression model with cluster as a random effect. An analogous model was used to analyse incident VL cases as a secondary outcome.
Findings: The odds for seroconversion were reduced in the individuals living in intervention clusters by 10% compared to individuals in control clusters (OR=0.90; 95% CI: 0.47, 1.73), the effect was however not significant. Similarly, LNs reduced the odds of VL by 11% (OR=0.89; 95% CI 0.57, 1.40), but the effect was, again, not statistically significant.
Interpretation: There is no evidence that LNs have a protective effect against *L. donovani* infection in VL endemic villages. VL control programs in the region should not rely on LNs only for sand fly control.

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Infection Patterns and Genetic Diversity of Cache Valley Virus Among Mosquitoes Collected in Connecticut

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Cache Valley virus (CVV; Family *Bunyavidae,* Genus *Orthobunyavirus*) is transmitted by multiple mosquito species in different regions of North America. To identify potential mosquito vectors and characterize major features of CVV transmission in Connecticut, we screened mosquitoes collected during the statewide surveillance program from 1997-2008 for viral infection in cell culture. In addition, CVV isolates were sequenced and compared by phylogenetic analysis to track transmission patterns of viral variants in this region. A total of 116 CVV isolations were recovered from 16 different mosquito species collected from late August to early October throughout the state. The virus was most frequently detected in *Anopheles punctipennis* (36 isolations) followed by *Ochlerotatus trivittatus* (14), *Oc. canadensis* (13), and *Aedes cinereus* (12). The number of virus isolations fluctuated annually with major increases in 1998 (22 isolations), 2003 (72), and 2008 (13) and relatively few (≤4) or no isolations during intervening years. Phylogenetic analysis revealed that viral genetic variation was limited and lacking obvious geographic structure but Connecticut strains could be delineated into three well-supported clades that persisted over multiple years of sampling. One of these clades included a virus that was previously isolated from a fatal human case in North Carolina. Together, these results implicate *Anopheles punctipennis* as an important vector of CVV in Connecticut where the virus is transmitted intermittently from late summer-early fall. This periodicity corresponds to the transmission of three dominant genetic clades of CVV that are broadly distributed throughout the state.

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Seasonal Influenza Vaccine and Protection against Pandemic (H1N1) 2009 Infections among US Military Personnel

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Context: A novel A/H1N1 virus is the cause of the present influenza pandemic; vaccination is a key countermeasure, however, no specific vaccine is available and data assessing vaccine effectiveness (VE) against Pandemic A/H1N1 2009 is lacking.
Objective: Assess the effectiveness of seasonal vaccines against clinically-apparent, laboratory-confirmed Pandemic (H1N1) 2009 infections.

Design, Setting and Participants: Surveillance of influenza-related medical encounter data of military service members stationed in the United States during the period of April-August 2009 with comparison of Pandemic (H1N1) 2009 confirmed cases and date-matched controls.
Main Outcome Measures: Crude odds ratios (OR) and VE estimates for immunized versus non-immunized were calculated as well as adjusted OR (AOR) controlling for gender, age, number of prior vaccinations, length of military service, and vaccine type (inactivated-TIV versus live attenuated-LAIV).
Results: For the period 20 April to 15 August 2009, 541 cases of Pandemic (H1N1) 2009 infections were reported; 423 (78%) among males and one-half (58%) among those under 25 years of age. Overall VE for service members was found to be 28% (95% CI, 1 to 48%). Immunization with prior season's LAIV (VE=42%, 95% CI, 18 to 59%), but not TIV (VE=23%, 95% CI, -9 to 46%) was found to be protective. Irrespective of vaccination status, increasing length of service (AOR=53% for 2 or more years, AOR=44% for 1-2 years, compared to those with less than 1 year) and age (AOR=0.19 for older than 39 years, AOR=0.22 for 30-39 years, 0.61 for 25-29 years, compared to those less than 25 years) was significantly associated with decreased odds of being a case. VE was lower in those having received 4 previous doses (VE=42%) compared to 3-dose (VE=45%), 2-dose (VE=45%), and one-dose recipients (VE=60%).
Conclusions: Immunization with LAIV, but not TIV, seasonal influenza vaccine in 2008-09 was associated with protection against clinically-apparent, laboratory-confirmed Pandemic (H1N1) 2009 infection. Number of prior vaccine doses in last 5 years (2004-09) was found to be inversely associated with protection, however, length of military service and increasing age were independently associated with a protective effect. Cross-protective immunity as a result of natural influenza infections, may play a role in conferring a certain degree of "immunological priming".

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The efficacy of different bed net against phlebotomine sand flies in Andean Peru.

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In Peru, the sand flies *Lutzomyia verrucarum* and *Lu. peruensis* are vectors of bartonellosis and leishmaniasis. Both species have anthrophilic and endophilic tendencies that are of public health and DoD importance. The primary objective of this study was to evaluate the comparative efficacies of DoD bed nets and new LLIN bed nets in preventing sand flies from penetrating the net.
The study was conducted in Jaguas (9° 24' S and 7° 35' W), Department of Ancash, Peru. Sand fly collections were performed from November 2007 to July 2008 using CDC light traps located inside the different bed nets. A modified Latin square design was used with 10 treatments within 10 randomly selected houses during a period of 10 days. This design was repeated 6 times. The treatment net and a control were set up in different rooms of the same house.
The LLIN bed nets used in this study consisted of Olyset® impregnated with permethrin and PermaNet® (mesh sizes 156, 232 and 270) impregnated with deltamethrin or untreated. The DoD nets used consisted of the Insect Net Protector (NSN) and the Self-Supporting, Low-Profile net (green and brown). A total of 5,232 sand flies were collected in 60 collections per treatment. Of these, 66 flies were collected inside the treated nets; *Lu. peruensis* (70.8%) was the predominant species, followed by *Lu. verrucarum* (28.8%) and *Lu. noguchi* (0.4%).
Fewer flies were collected in treated LLIN nets (range 3-9 flies) compared with untreated LLIN nets (17-23 flies). No sand flies were collected in any of the DoD nets.

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Ecological niche modelling of malaria vector habitat and mapping of malaria risk in northern Tanzania

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Malaria transmission is spatially heterogeneous, with foci of transmission occurring in close proximity to malaria vector habitat. Targeting control interventions in transmission hotspots helps maximize the effectiveness of limited malaria control resources. Ecologic factors that determine the suitability of habitat for malaria vectors can be identified by satellite remote sensing, providing means to model vector distributions across wide geographic areas. Using a maximum entropy approach, we modelled the ecological niches of the three major vector species in northern Tanzania over a 94,000 km^2 area by relating vector point occurrences to high resolution environmental and climatic data. Seasonality of precipitation, maximum temperature of the warmest month and land cover contributed the most to models for *An. arabiensis* and *An. funestus* s.l. (model AUC 0.989 and 0.991, respectively) while precipitation of the coldest quarter, land cover and altitude were most important for *An. gambiae* s.s. (model AUC 0.997). Resulting estimates of area of suitable vector habitat within 1.5 km interacted with altitude to substantially improve regression model predictions of community-based prevalence of malaria in children, providing a strong validation of niche modelling outputs. After accounting for spatial autocorrelation, addition of area of vector habitat to the model accounted for 66% of the residual variation in malaria prevalence compared to the model with altitude alone (model $r^2 = 0.83$ vs. 0.50). We then applied the validated regression equation using high resolution spatial data to predict malaria prevalence across the study region. The novel application of landscape-scale ecological niche modelling of malaria vectors using purpose-built high resolution land cover data identifies malaria hotspots at a

practical scale for targeting malaria control interventions. This strategy may be particularly relevant in the East African highlands as rapidly changing land uses and climate may exacerbate recent malaria resurgence.

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Expression and characterization of recombinant Plasmepsin X, a P. falciparum aspartic protease

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Malaria is among the most devastating infectious diseases affecting developing countries. Plasmodium, the parasite that causes malaria, must invade multiple tissue types in its vertebrate and invertebrate hosts. Numerous proteins secreted by Plasmodium are vital for its survival and successful propagation throughout its complex life cycle. Plasmepsin X (PM X) is an aspartic protease believed to play an essential role in the invasiveness of Plasmodium falciparum, the species responsible for the most lethal form of human malaria. The objective of this project is to express active, recombinant protease. Recombinant PM X was initially expressed in E. coli as insoluble protein but failed to generate active protease. Soluble protease has been subsequently expressed in a wheat-germ expression system. We will determine the conditions for activity for this protein as well is its substrate. Expression of active, recombinant PM X will facilitate biochemical understanding of its role in malaria transmission and could lead to a novel anti-malarial drug.

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A role for the C-terminus of arenavirus NP in its incorporation into Z-driven virus-like particles

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Members of the Arenaviridae family, such as Lassa, Junin, Machupo, and Guanarito hemorrhagic fever viruses, cause significant public health problems in areas of South America and Africa. No protective vaccines are available to control arenavirus infections, and the lone effective antiviral drug, ribavirin, is only effective when given early in the course of clinical illness and is associated with significant side effects. To identify targets for drug development and vaccines, it is important to understand the lifecycle of arenaviruses.
Arenaviruses are enveloped, negative-strand RNA viruses. The viral matrix protein, Z, facilitates virus-like particle (VLP) formation in several arenaviruses, including Lassa virus. However, the mechanism by which viral ribonucleoprotein complexes are incorporated into viral particles is poorly understood. Here, we generated plasmids for the expression of the Z and NP proteins of Mopeia virus, which is closely related to Lassa virus but does not cause disease in humans. Expression of Mopeia virus Z protein with wild-type Mopeia virus NP protein resulted in the highly selective incorporation of NP protein into Z protein-induced VLPs. We also found that Z protein actively promoted NP association with cellular membranes, suggesting that the association between NP, Z, and the membranes may facilitate efficient NP incorporation into VLPs. Further, by employing a series of NP deletion constructs and testing their VLP incorporation, we demonstrate an important role for the C-terminal half of NP in VLP incorporation.

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Bacterial Contamination Associated with Sushi Purchased from Different Venues

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The popularity of sushi has increased potential ingestion of seafood-associated pathogens. This survey evaluated bacterial contamination of sushi purchased at 8 venues in the Washington DC area: 2 sushi restaurants, 2 Asian restaurants, 2 gourmet supermarkets, and 2 general supermarkets. Sushi purchased on Monday and Wednesday included 4 types from each venue: (cooked: shrimp & crab) and (raw: tuna & salmon). 60 total samples were collected. A slurry of a 5 mm slice through each sample (including fish, rice, seaweed wrapper) and 5 mL of fastidious broth was made using a tissue grinder. A 0.001 mL loop inoculated slurry onto blood, MacConkey and Campy-blood agar. 59/60 (98%) of samples yielded bacterial growth, the median 16,000 colonies/mL was broken into quartile ranges of scant: 1-7000; low: 8-16000; moderate: 17-45000 and heavy: >45000 colonies/mL.
Grocery stores demonstrated greater numbers of gram pos and gram-neg bacteria and a greater variety of bacterial species than restaurants. There were more species (mean 2.2 vs 1.8 species) and higher colony counts (10 vs 5 samples with heavy contamination) from sushi purchased on Monday vs Wednesday. Cooked shrimp and crab demonstrated more bacterial contamination than raw salmon and tuna and had a wider variety of bacterial species. Bacteria encountered were more consistent with contamination from foodhandlers and surfaces (coagulase neg *Staph* sp, enterococci, enteric & environmental gram neg rods and yeast) than those associated with raw seafood. *Shigella flexneri* was the only diarrhea associated pathogen isolated and was encountered on one shrimp sample.
These data suggest that there may be significant bacterial contamination of sushi from a variety of venues. The bacterial strains encountered imply contamination is not due to seafood but to inadequate hygiene from food handlers and the surfaces used for

preparation. Although many of the species encountered are not associated with disease, the finding of enteric gram negative rods, including *Shigella,* suggests that diarrhea may be linked to eating sushi.

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Modeling the suppression of Aedes aegypti populations using releases of transgenic mosquitoes with conditional female lethality

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Genetic strategies have opened new avenues for the control of disease-vectoring mosquitoes such as Aedes aegypti. In particular, the use of transgenic mosquitoes carrying a conditional female lethality gene has been proposed as a method of population suppression. When these transgenic individuals are released into a resident population and crossed with wild-type mosquitoes, their female offspring are not viable, thus lowering the vector population. Here we use Skeeter Buster, a detailed, spatially-explicit model of Ae. aegypti populations, to assess the outcome of various types of transgenic releases. We parameterize the model based on data from the city of Iquitos, Peru, including real weather data and a representative distribution of water-filled containers. We compare releases of transgenic male adults, pupae or eggs into the resident population. We consider different spatial release patterns: a homogeneous release in every house or a release in 10% of houses selected at random or uniformly distributed. We show that the homogeneous release of adults requires the lowest number of transgenic insects, and that the release of immature stages can result in successful control if the release sites are uniformly distributed. Finally we quantify the mosquito release rates that need to be sustained following local suppression in order to prevent reestablishment of the vector population from immigration of wild mosquitoes.

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Evolutionary epidemiology of Andes virus: Bayesian analyses reveal increase in the number of infections in rodents and humans congruent with increase in HCPS cases

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Evolutionary epidemiology is a multidisciplinary area that integrates ecology, evolutionary biology, and public health. It allows understanding the mechanisms of transmission and dynamics of diseases at different spatial and temporal scales. Hantaviruses are single stranded negative sense RNA viruses that can be transmitted to humans through rodent excreta and secretions. In the Americas, hantaviruses (Bunyaviridae) are the etiological agents of hantavirus cardiopulmonary syndrome (HCPS). In Chile, Andes virus ANDV is responsible for all cases of HCPS, and Oligoryzomys longicaudatus (Sigmodontinae) is the main reservoir. This rodent occurs in Chile along a wide latitudinal range that spans contrasting geographic features and landscapes. ANDV epidemiology is complicated because person-to-person transmission has been well documented in Argentina and Chile. Using molecular sequences, we assessed phylogeographic structure and spatial genetics of ANDV in Chile. ANDV is segregated into genetic lineages that correspond to the limits of ecogeographic regions. Using Bayesian Skyline Plot analyses, we show that the effective number of infections through time increased exponentially from 1999, reaching a peak during the years 2001 to 2002, after which the growth rate decreased, congruent with a peak in the number of HCPS cases. We also compare the anti-ANDV antibody prevalence of O. longicaudatus across the latitudinal gradient, and provide an assessment of the distribution of incident and fatal cases of HCPS in Chile. We discuss the importance of combining information from host-virus population structure and epidemiology to gain insights into the transmission and persistence of infectious diseases.

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Estimating a repellent's potential to reduce malaria in communities

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To provide a means for estimating the epidemiological efficacy of repellents in poor communities, a mathematical model was developed. In order to demonstrate the functions of this computational tool, the performance data of a highly effective, low cost repellent were used. The model shows that mass distribution of a repellent with >98% efficacy and user acceptance would suppress new malaria infections to levels significantly lower than those achieved with insecticide treated nets (ITNs).

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Origin, phylogeographic structure and spatial genetics of Chagas disease vector (Triatoma infestans) in Chile

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Chagas disease is one of the most important vector-borne diseases in Latin America with a large number of people infected during its 100 years of history. The disease is caused by the flagellate protozoan Trypanosoma cruzi, which in South America, is commonly transmitted to humans by the kissing bug Triatoma infestans (Reduviidae, Triatominae). This species occurs in Chile along a latitudinal range (18-34° S) that spans northern desert, semi-desert, and central Mediterranean areas. Control programs have eliminated T. infestans from large domestic areas; however, recent sylvatic foci have been reported in central Chile. These findings highlight the necessity for new studies characterizing both domestic and sylvatic populations in endemic areas of the country. Using molecular sequences, we assessed phylogeographic structure and spatial genetics of T. infestans in Chile. Using a Bayesian framework, we also assessed the origin of Chilean populations and the divergence time of T. infestans from other Triatoma species. Phylogenetic analyses showed three major groups in Chile: the northern group clustered with Peruvian and Bolivian haplotypes, and two North-central groups that clustered with Argentinean and Uruguayan haplotypes. These results suggest multiple origins of Chilean kissing bugs estimated 0.16 - 0.36 Mya. Landscape genetic interpolation revealed higher genetic distances in samples from northern Chile. T. infestans would have separated from other Triatoma species around 0.6 Mya. We discuss the implications of the Chilean sylvatic populations as potential sources of domiciliation, and highlight the relationship between public health and evolutionary approaches. Fondecyt 1070960.

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CUTANEOUS LARVA MIGRANS: A RARE CAUSE OF SKIN RASH IN THE UNITED STATES.

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Introduction: Cutaneus larva migrans (CLM) also known as creeping eruption due to the larva of parasitic worm is commonly encountered in tropical climate worldwide but rarely diagnosed in temperate climates.
Case: 59 years old man from South Carolina presented with a four week history of maculopapular rash on the left groin and hip area. The rash was intensely pruritic and later painful. He had been working long hours on his lawn at the time the rash appeared. He had no fever, no purulent discharge from the rash and no history of similar rash in the past. He owned pets, had no recent travel, sick contact and no rash in any family member. He had completed a course of valacyclovir, diflucan and sulfamethoxazole with no improvement. His vitals signs were normal. Skin examination showed diffuse patchy maculopapular rash with threadlike erythematous extensions. The lesions were not ulcerated and there was no discharge from the lesions. Blood studies were normal except eosinophilia of 4.4%. There was no growth from blood cultures. Skin biopsy showed superficial and deep perivascular inflammation with numerous interstitial eosinophils. There was a focal lesion with acute inflammation consistent with arthropod bite reaction. Periodic acid schiff stain for fungal elements was negative. CLM was diagnosed and treated with oral albendazole 400mg daily for 7days. On this regimen, the rash cleared rapidly with resolution of the pruritus. Outpatient follow-up confirmed complete remission of the condition with no subsequent recurrence.
Discussion: The diagnosis of CLM is clinical, based on the typical history of exposure to soil or sand, the appearance of the rash and geographic area with tropical climate. Individuals affected by CLM are involved in outdoor activities where there is contact of the unprotected skin with contaminated soil which facilitate the penetration of the exposed skin by the larva of parasites especially *Ancyclostoma* spp, *Ascaris* spp and *Strongyloides*. The clinical presentation is a maculopapular rash with surrounding erythema and a winding threadlike subcutaneous trail of reddish brown inflammation described as serpiginous. Lesions are responsive to oral albendazole, ivermectin or topical thiabendazole.
Conclusion: A high index of suspicion on the part of the clinician coupled with a detailed occupational and recreational history is needed to make the right diagnosis and initiate appropriate anti parasitic therapy.

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"Malaria complicated by Plasmodium vivax in Iquitos, Peru: A case report"

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BACKGROUND: World-wide, malaria continues to be a significant public health problem. In Peru, the majority of malaria cases are caused by *Plasmodium falciparum* and *Plasmodium vivax*. However, it is unusual to find severe/complicated *Plasmodium vivax* infections. Around Iquitos, Peru, there are many communities where nutrition and quality of life are sub-optimal. The cases presented here are from these communities. Although the malaria case rate has decreased over the past few years, this area continues to have constant transmission.

CASE PRESENTATION: A 3 month old male child (a twin), presented with seven days of fever, chills, and paleness, as well as with five days of a dry exigent cough, hyporexia, poor suction reflex, listlessness, and dehydration. The patient had a high density of *P. vivax* trophozoites (8,068 parasites/ul) and had a *P. vivax* gametocyte density of 408 parasites/dl. The patient exhibited a hematocrit of 20% PCV, leucopenia (5,000/mm^3), 200/mm^3 band neutrophils, and a low platelet count (29,000/mm^3). High parasitemia coupled with alterations in the three blood cell types indicated that the patient was septic. The patient was treated with chloroquine for 7 days, and also received treatment for pulmonary edema with penicillin and cloramphenicol for 9 days and dexamethasone for 5 days. At the patient's control visit, 7 days after being diagnosed with and treated for *P. vivax* malaria, the patient continued to have trophozoites (71 parasites/ul). The patient was re-treated and no parasites were observed after the second treatment.
CONCLUSION: A complicated case of *P. vivax* malaria is presented in this study. As in most recent cases there are no hospital records for these patients. Here we observed one of the rare severe *Plasmodium vivax* cases reported in this region of Peru. We recommend closely monitoring of *Pasmodium vivax* cases to determine the overall prevalence of complicated and severe malaria.

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Towards Better Tuberculosis Diagnostics: a Systems Biology Approach

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Background:
Host genetic response is important in determining outcome following exposure to *M. tuberculosis* (TB). Current diagnostic tests for TB are suboptimal. We sought to determine the performance of global gene expression profiling in differentiating types of mycobacterial exposures.
Methods:
Total RNA was extracted from peripheral blood mononuclear cells from 4 groups of volunteers: healthy unexposed, BCG vaccinated, latently infected (LTBI), and active TB disease (ATB). Robust multichip averaged normalized data sets generated on Affymetrix 133 Plus 2.0 GeneChips were analyzed using Significance Analysis of Microarrays. Prediction Analysis of Microarrays with Program R (PAM-R) and principal component analysis (PCA) were used to determine if signature gene sets distinguished the four groups. Pathway analysis was performed using gene enrichment analysis against the Molecular Signatures Database of the Broad Institute.
Results:
A 127 gene signature set was identified that distinguished persons with BCG, LTBI, and ATB with 100% accuracy, independent of the method used (PAM-R vs. PCA). Hierarchical cluster analysis revealed gene expression patterns unique to each group. Functional classification of significantly differentially expressed genes in ATB indicated a preponderance of up-regulated genes with functions related to autoimmune dysfunction, inflammation, and γ-interferon response. Groups were also distinguishable based on the number and type of up-regulated pathways. In the ATB group, 18 of the up-regulated pathways involved interferon response, compared to 2 in the LTBI and 1 in the BCG group.
Conclusion:
A systems biology approach using genome-wide transcription profile analysis of persons with LTBI, BCG, and ATB may be useful to develop molecular biomarkers that can predict outcome following TB exposure and may eventually outperform current diagnostic tests.

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Dehydroepiandrosterone and dexamethasone Delay Progression of Experimental Leishmaniasis in Mice

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DHEA and cortisol serum concentrations are low in patients with diffuse leishmaniasis. To determine if DHEA or dexamethasone can modify the development of leishmaniasis, susceptible BALB/c male mice were infected with *Leishmania mexicana* at a dose capable of giving rise to diffuse leishmaniasis. Eleven months after infection, when lesions extended to the tail, mice were supplemented with DHEA, dexamethasone, or both over three months. All supplemented mice, experienced delays in increases of lesion size; however, lesion size increased again after suspending DHEA or dexamethasone. Furthermore, the mortality rate was lower only in the DHEA-treated group. In conclusion, during murine experimental leishmaniasis by *Leishmania mexicana,* both DHEA and dexamethasone prevent increases in lesion size and retard diffusion of leishmaniasis. Only DHEA reduces mortality of infected mice.

2975

A study of obstacles and opportunities for gender equity in tuberculosis control.

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Background. Female gender may be associated with reduced likelihood of TB diagnosis and successful treatment, so we characterized gender-related barriers to TB control.
Methods. We investigated experiences and attitudes relating gender to TB in 17 shantytowns near Lima, Peru. Epidemiological analysis characterized TB-related gender differences. These were then investigated using the grounded theory approach amongst key TB control stakeholders in 22 semi-structured interviews and in 4 focus group discussions with 26 TB patients and 17 health care workers.
Results. We actively screened 3599 people heavily exposed to TB and diagnosed TB disease in 141 of them, with similar frequency in both genders (51% women). In contrast, standard passive case finding diagnosed fewer women (40% of 1259 patients; P<0.01). In another shantytown, adherence to TB therapy in 262 patients was more likely to be interrupted in women (adjusted odds ratio 6.7; P=0.02). Despite these TB-related gender inequalities, in qualitative studies the TB program was perceived not to be gender discriminatory and to provide equal TB care to men and women. This contrasted with stereotypical gender roles and a commonly expressed belief amongst patients and healthcare workers that female health inherently has a lower priority than male health. This belief was principally associated with men's predominant role in the household economy and limited employment for women in this setting. Women were also generally reported to experience the adverse psychosocial and economic consequences of TB more than men.
Conclusions. Standard TB passive case-finding under-diagnosed women, who were also more likely to suffer treatment interruptions. This gender inequality was associated with a common perception that women's TB care was of secondary importance to that of men. This reflected societal gender values and was despite apparent gender equality in care provision. The greatest opportunities for improving women's TB care appear to be in improving social, political and economic structures, more than TB program modification.

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Protection against Plasmodium vivax malaria infection by G6PD deficiency

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Background: The most common form of malaria outside Africa, Plasmodium vivax, is more difficult to control than P. falciparum because of the latent liver hypnozoite stage which causes multiple relapses and provides an infectious reservoir. Current anti-hypnozoite drugs can cause significant haemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency which restricts usage in low-resource settings. The African (A-) G6PD deficiency confers partial protection against severe P. falciparum but the effect of the deficiency on vivax malaria has not been established. If the deficiency confers substantial protection against P. vivax, this could reduce the risk of deploying anti-relapse drugs and further explain the geographical distribution of G6PD deficiency in human populations. We tested the hypothesis that G6PD deficiency is protective against vivax malaria infection.
Methods and findings: A case-control study design was used amongst Afghan refugees in Pakistan. The frequency of phenotypic and genotypic G6PD deficiency in individuals with vivax malaria was compared against controls who had not had malaria in the previous two years. Phenotypic G6PD deficiency was less common amongst cases than controls (cases: 4/372 (1.1%) vs. controls 42/743 (5.7%); adjusted odds ratio (AOR) 0.18 [95% confidence interval: 0.06-0.52], p=0.001). Genetic analysis demonstrated that the G6PD deficiency allele identified (Mediterranean type) was associated with protection in heterozygous (AOR = 0.39 [95%CI: 0.16-0.98], p=0.045) and homozygous females and in hemizygous deficient males (AOR = 0.13 [95%CI: 0.02-0.97], p=0.047).
Conclusions: G6PD deficiency confers significant protection against vivax malaria infection whether measured by phenotype or genotype. The risk-benefit assumptions which restrict deployment of anti-hypnozoite drugs are not supported by this finding which also indicates a role for vivax malaria in the retention of the G6PD deficiency trait in human populations.

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Heterologous prime-boost vaccination with AdCh63 ME-TRAP and MVA ME-TRAP can induce sterile immunity to sporozoite challenge in healthy malaria-naive volunteers

Geraldine A. O'Hara[1], **Christopher J. Duncan**[1], Katie Ewer[1], Arturo Reyes-Sandoval[1], Anna Goodman[1], Nick Edwards[1], Katharine Collins[1], Ian Poulton[1], Steven Aston[1], Rosalind Rowland[1], David W. Porter[1], Simon Correa[2], Pru Bird[3], Eleanor Berrie[3], Andrew M. Blagborough[4], Robert E. Sinden[4], L. Siani[5], Stefano Colloca[5], Ricardo Cortese[5], Alison Lawrie[1], Alfredo Nicosia[5], Sarah C. Gilbert[1], Adrian V. Hill[1]
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Introduction
Chimpanzee adenoviruses are novel candidate vectors for vaccines that need to induce strong cellular immunity in populations with widespread exposure to human adenoviruses, and we have recently found these to be safe and immunogenic in a phase I malaria vaccine trial in healthy volunteers. Here we report the outcome of a phase IIa sporozoite challenge trial.
Methods
We vaccinated 8 healthy adult volunteers once with AdCh63 expressing thrombospondin-related adhesion protein with a multiple-epitope string (AdCh63 ME-TRAP) and boosted once 8 weeks later with modified vaccinia virus Ankara (MVA) expressing ME-TRAP. We also vaccinated 10 volunteers with AdCh63 ME-TRAP alone. All vaccinated volunteers underwent a standard 5 bite sporozoite challenge 2-3 weeks following their last vaccination, together with 6 unvaccinated control volunteers.
Results
The vaccines were well tolerated with a good safety profile. The prime-boost group showed unprecedented T cell immunogenicity with mean γ-interferon ELISPOT responses exceeding 2500 SFU per million PBMCs. 25% of these volunteers but no controls were completely protected from clinical malaria. Analysis of humoral and cellular immune responses showed that specific cellular responses defined by flow cytometry, but not humoral immune responses correlated with protective efficacy.
Discussion
Simian adenovirus-MVA based viral vector vaccines are exceptionally immunogenic and can successfully engender sterile protection in malaria naive volunteers.

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Discussion
Simian adenovirus-MVA based viral vector vaccines are exceptionally immunogenic and can successfully engender sterile protection in malaria naive volunteers.

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Oral activated charcoal as a potential adjunct therapy for severe malaria

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Background: Safe, cheap and effective adjunct therapies preventing the development of, or reducing the mortality from, severe malaria could have considerable and rapid public health impact. Oral activated charcoal (oAC) is a safe and well tolerated treatment for acute poisoning, more recently shown to have significant immunomodulatory effects in man. In preparation for possible efficacy trials in human malaria, we sought to determine whether oAC would i) reduce mortality due to experimental cerebral malaria (ECM) in mice, ii) modulate immune and inflammatory responses associated with ECM, and iii) affect the pharmacokinetics of parenteral artesunate in human volunteers.
Methods / Principal Findings: We found that oAC provided significant protection against *P. berghei* ANKA-induced ECM, increasing overall survival time compared to untreated mice ($p<0.0001$; hazard ratio 16.4; 95% CI 6.73 to 40.1). Protection from ECM by oAC was associated with reduced numbers of splenic TNF^+ $CD4^+$ T cells and multifunctional $IFN\gamma^+TNF^+$ $CD4^+$ and $CD8^+$ T cells. Furthermore, we identified a whole blood gene expression signature (68 genes) associated with protection from ECM. To evaluate whether oAC might affect current best available anti-malarial treatment, we conducted a randomized controlled open label trial in 52 human volunteers (ISRCTN NR. 64793756), administering artesunate (AS) in the presence or absence of oAC. We demonstrated that co-administration of oAC was safe and well-tolerated. In the 26 subjects further analyzed, we found no interference with the pharmacokinetics of parenteral AS or its pharmacologically active metabolite dihydroartemisinin.
Conclusions / Significance: oAC has an ideal profile for use in developing countries, being inexpensive, well-tolerated at high doses and requiring no sophisticated storage. These characteristics, together with the data reported here, make oAC an exciting candidate for adjunct therapy to reduce mortality from severe malaria, or for immediate treatment of suspected severe malaria in a rural setting.

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Mapping trachoma risk in Southern Sudan

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Trachoma, caused by the bacterium *Chlamydia trachomatis*, is the main cause of infectious blindness worldwide and thought to be one of the main causes of blindness in Southern Sudan. Transmission of *C. trachomatis* in ocular discharges can be direct, or via flies, and is associated with poverty and related issues of poor hygiene and sanitation. Given the probable environmental drivers of transmission, spatial prediction of trachoma distribution using geographical information systems and spatial statistics is plausible, but has not been previously attempted. Bayesian geostatistics provides a platform for spatial prediction that can incorporate environmental covariates and spatial autocorrelation in the data, and permits assessment of uncertainties in resultant spatial predictions. We used this approach to develop a model to predict the risk of trachoma infection in Southern Sudan. Trachoma prevalence data were obtained from cluster random field surveys in 80 geo-referenced communities. Logistic regression models were developed in a Bayesian framework using active trachoma infection (trachomatous inflammation follicular and/or trachomatous inflammation intense) in 2748 children aged 1-9 years as the outcome, incorporating fixed effects for age, rainfall, land cover and tribe and geostatistical random effects describing residual spatial variation. The model predicts the risk of active trachoma infection throughout Southern Sudan, as well as the associated uncertainty of such prediction. Risk was negatively associated with age and rainfall, but positively associated with forested areas. Sedentary agriculturalist tribes were at lower risk than pastoralist tribes. Predicted risk was high in arid areas in the central, northern and south-eastern zones, and lower in the wetter areas in the south-west. These risk maps present useful tools to target scarce resources to baseline surveys and subsequent interventions, as well as to estimate the national trachoma burden and the resources required to meet the 2020 target of eliminating blinding trachoma from Southern Sudan.

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Efficacy and Pharmacokinetics of SCYX-7158 (AN 5568): a Novel and Potent Oxaborole-6-Carboxamide Selected as a Pre-Clinical Candidate for Once-Daily Oral Treatment for Stage 2 Human African Trypanosomiasis.

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SCYX-7158, a 3,3-dimethyloxaborole-6-carboxamide, is distinguished from earlier trypanocidal oxaboroles by enhanced pharmacokinetic and CNS disposition properties allowing for a once per day (QD) oral dosing regimen at a markedly lower efficacious dose in a Stage 2 murine Human African Trypanosomiasis (HAT) model. The discovery of SCYX-7158 was achieved through application of integrated lead optimization strategies across medicinal chemistry, parasitology and pharmacokinetic disciplines. SCYX-7158 is active in vitro against relevant strains of Trypanosoma brucei, including T. b. rhodesiense and T. b. gambiense (IC50 values = 0.18 - 0.98 μM) and is efficacious in both Stage 1 and Stage 2 murine HAT models. Physicochemical and in vitro ADME properties of SCYX-7158 are consistent with the compound being orally available, metabolically stable, readily CNS permeable and with low risk for drug-drug interactions. In an ongoing murine Stage 2 study, SCYX-7158 is effective orally at doses as low as 12.5 mg/kg (QD x 7 days). In vivo pharmacokinetic characterization of SCYX-7158 demonstrates that the compound is highly bioavailable in rodents (F >50%, mouse), has low intravenous plasma clearance (89 mL/hr/kg - less than 5% liver blood flow), a 24 hr elimination half-life and a volume of distribution (Vdss = 1.7 L/kg) that indicates good tissue distribution. Most importantly, brain exposure of SCYX-7158 is high, with Cmax > 10 ug/mL and AUC0-24hr > 100 μg*hr/mL following a 25 mg/kg oral dose. Furthermore, SCYX-7158 readily distributes into CSF and crosses the blood-testicular barrier to achieve therapeutically-relevant concentrations in potential trypanosomal sanctuary sites. Early PK-PD and comparative pharmacokinetic data (rat and non-human primates) will be presented. Based on these properties, which promise lower rates of recrudescence than with current standard of care, SCYX-7158 has been selected as a pre-clinical candidate for treatment of Stage 2 HAT.

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Mobile phone usage data as a tool for malaria elimination planning

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Zanzibar has commissioned a feasibility assessment to help inform on whether to move to a malaria elimination campaign. Following intensive control, historically low levels of transmission have refocused attention on imported malaria. Anonimised mobile phone records provide a valuable data source for characterising human movements in areas that are typically data-sparse without compromising the privacy of phone users. Such data, in combination with maps of *P. falciparum* endemicity, were used to characterize the patterns of parasite carrier movements and rates of malaria importation by Zanzibar residents.
Records encompassing 3 months of complete mobile phone usage were obtained from the Zanzibar Telecom (Zantel) mobile phone network company, including the dates of all phone usage by 770,369 individual anonymous users. Each individual call and message was spatially referenced to one of six Tanzanian regions. Information on the numbers of Zanzibar residents travelling to the mainland, locations visited and lengths of stay were extracted. Spatiotemporal data on *P. falciparum* transmission intensity and seasonality, combined with malaria transmission models, enabled estimates of parasite importation rates to be made.
No long distance travel was apparently undertaken by 88% of Zanzibar residents. Of those who travelled, the vast majority of trips were estimated to be of less than five days in length, and to the Dar Es Salaam region. Data on total infection numbers in Zanzibar combined with mathematical models enabled informed estimation of transmission exposure and average estimates of between 1 and 12 imported infections per 1000 residents per year. These also showed that the majority of trips made posed a relatively low risk for parasite importation, but risk groups visiting higher transmission regions for extended periods of time could be identified. These analyses represent the first quantification of the vulnerability of a region to imported infections, providing information that is central to assessing the feasibility of malaria elimination.

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The importance of characterizing communities in terms of their hematological and biochemical parameters- the case of Kintampo in Central Ghana

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The Kintampo Health Research Centre with its study area based in central Ghana has been carrying out clinical trials in the past eight years. Screening of study participants, monitoring of their safety and management of adverse events during the clinical intervention trials has been core, though values being used as reference have been those obtained from a completely different population and may be different from values in our study area. This study aimed at establishing hematological and biochemical reference values for the communities in the study area.
A random list of communities and a list of reference individuals were generated from the study area using Visual FoxPro software. Following consent, a questionnaire containing demographic and clinical information was completed for each selected individual and eligibility of each participant was determined by a study clinician. Fasting venous blood samples were collected into EDTA and serum separator tubes (SST) for Haematology and Clinical Chemistry tests. SST samples were centrifuged and serum collected for clinical chemistry analysis on the Vital Scientific Selectra E Clinical Chemistry analyzer (Dieren, The Netherlands). EDTA samples were analyzed using the ABX Micros 60 Haematology analyzer (Montpellier, France). Data was double-entered using the Visual FoxPro software. Reference values for Hemoglobin (HB), Total white blood cells (WBC), Platelets, Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Total Bilirubin (BIT) , Urea and Creatinine were determined using Stata Statistical software. Preliminary results from 350 children under 5 years of age using the 2.5th and 97.5th percentiles indicate a Hemoglobin concentration of 7.1-12.8g/dL, WBC count : 4.5-17.0 x109/L, Platelets: 86-650 x109/L, RBC: 3.05-5.50 x1012/L; ALT: 6-51 U/L, AST: 21-72 U/L, BIT: 2.0-22.0 µmol/L, Urea: 0.5-4.5 mmol/L and Creatinine: 13-59 µmol/L
These preliminary results show significantly lower values for hemoglobin, RBC and urea with higher values for ALT and AST. If the results obtained so far are to persist, then there will be the need to change the reference values used in future in order to be more applicable in the study area.

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PAEDIATRICS CHAGA'S DISEASE IN NON-ENDEMIC AREA.

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INTRODUCTION
The increasing presence of Chagas disease in non-endemic countries, have an impact in public health policies in Europe and United States. Congenital transmission in these countries has already been reported and is one of the most important potential routes of transmission. We aimed to establish the prevalence of *Trypanosoma cruzi* infection in Latin American pregnant women and their congenital transmission rate in Barcelona and also the early diagnose in children is crucial for the proper evolution without sequelae.
OBJECTIVES
The aim of this study is to describe the cases of children with Chagas Disease attended at our Imported Pathology Unit during the last years (2003-2008)
MATERIAL AND METHODS
All patients coming from endemic areas, and newborns from Chagas seropositive pregnant women, were studied after informed consent. Two ELISA tests were performed for diagnosis and PCR was done to seroreactive patients. We did parasitological exam and PCR to the newborns at birth, and ELISA test at birth and 8 months later. Epidemiological and clinical data was recorded in a program ad-hoc for this study. Data was analyzed with Stata 9.2. Confirmed cases received Beznidazole treatment (7-10 mg/kg/day during 60 days)
RESULTS
Screening was performed in 202 patients (156 immigrants and 46 born in Spain) 46 of them were seroreactive in both ELISA tests (22.7%) 31 was less than 12months of age. Vertical transmission was demonstrated in 5 children. The 15 infected children older than 12 months, were born in Bolivia. Follow up is in course in eight of the seroreactive patients. In order to establish the effectiveness of treatment, negative PCR and decreasing specific antibodies must be demonstrated in all studies patients.
CONCLUSIONS:
Our results demonstrate a seroprevalence for *T.cruzi* infection of 22,7% (46/202) 46 was born in Spain and the incidence of congenital infection was 10'7% (5/46) Chagas disease is an emergent infection in Spain that includes the risk of its vertical transmission. We strongly recommend the routinely screening for Chagas disease in non endemic countries in pregnant women coming from Latin America. All the patients showed clinically favourable responses to treatment, only two had anaemia. Protocols are required to detect early congenital and paediatric infection, in non endemic areas.

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Investigation of Uganda Tour Companions of the First U.S. Case of Imported Marburg Hemorrhagic Fever**,** 2009

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In January 2009, CDC identified the first US case of imported Marburg hemorrhagic fever (MHF) in a traveler who visited Uganda in December 2007. Exposure likely occurred during a visit to the "Python Cave," which is inhabited by bats, a presumed reservoir host of Marburg virus (MARV). The US patient reported that she traveled with 8 tour companions (TC) from Uganda, Belgium, the United Kingdom, and the United States. Public health agencies in these countries assisted CDC with the investigation. Our objectives were to describe common exposures, identify additional cases or evidence of prior MARV infection, and determine awareness of travel health risks and level of pre-travel health advice obtained by the TC. We administered a standard survey and offered testing for anti-MARV IgG antibodies by enzyme-linked immunosorbent assay. Surveys were completed by all 8 TC; all reported entering the cave and 6 (75%) reported climbing boulders to farther inside, as the US case did. However, none reported knowingly having direct contact with bats or bat guano/urine. One reported a mild illness 18 days after visiting the cave; a malaria test was negative and symptoms resolved within a few days. Five (63%) provided blood samples, including the TC who reported illness; none had evidence of prior MARV infection. Of the 6 (75%) who visited a clinic pre-travel, none received advice about health risks of bats or caves; nor did the park guide provide such information to the 8 TCs at the cave. While TC exposures were similar to those of the US patient, no evidence of MARV infection was detected among TC. Possible reasons include differences in routes of MARV exposure, infective doses, or host factors. Pre-travel healthcare providers should discuss itineraries with travelers, even with experienced international travelers, to identify health risks associated with certain activities. Specifically, travelers should be made aware of serious health risks, such as MHF in Africa and rabies and histoplasmosis worldwide, associated with bats and the enclosed areas they inhabit.

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Natural aestivation of *Anopheles gambiae* in the Sahel"

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Malaria remains a top public health priorities across Sub-Saharan Africa, where it is transmitted primarily by Anopheles gambiae s.s., An. arabiensis and An. funestus. Populations of these species exploit diverse environments including expansive dry savannahs and semi-desert areas, where surface waters, required for larval development, are scarce or totally absent for large part of the year. How do these mosquitoes survive the long dry season has remain an enigma for over 60 years. Although several studies found a few mosquitoes during the dry season, they could never determine if these mosquitoes survived throughout the long dry season (aestivation) or they 'migrated' from area where permanent breeding was ongoing. Here we show unequivocally that An. gambiae aestivates based on a mark-release-recapture experiment spanning the period from the end of one wet season to the beginning of the next. We found that within five days after the first rain, before a new generation of adults could be produced, mosquito abundance surged ten folds indicating that a substantial population was hidden locally until the first rain. Four days after the first rain, a marked female An. gambiae s.s. was recaptured. Initially captured, marked, and released at the end of the previous wet season, she has survived under natural conditions throughout the seven month long dry season. These results demonstrate that aestivation occurs in An. gambiae and explain how populations persist throughout the Sahelian dry season.

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Possible Role for Toll like Receptors in Interaction of Fasciola hepatica Excretory / Secretory Products with Monocite Cells

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Fasciola hepatica, the common liver fluke, causes widespread disease in farm animals as well as in man. Vaccines targeting crucial biologically active molecules secreted by the worms to facilitate survival in the host constitute a novel approach to control. There have been many attempts so far to prepare a protein vaccine against fascioliasis but they have not been succeeded in introducing a reliable vaccine on the market. The major problem linked with introducing protective immune response against F. hepatica is that the precise mechanisms of protection against this parasite have not been elucidated. It is well accepted that F. hepatica is able to immunomodulate its host secreting products (ES antigens) that often polarize the immune responses toward the Th2 end of the spectrum. These polarized immune responses may be associated with the development of chronic infections while avoiding the clearance by the host. Because the innate immunity constitutes the first line of defense against infections and molecules produced during innate immune responses stimulate and influence the nature of adaptive immune response, it's possible to hypothesize that the F. hepatica ES products (FhES) are responsible to interact with the cells of innate system resulting in a favorable immunological response that facilitate the parasite survival into the host. In the current study, we present data examining the interaction of total and molecular mass-fractioned ES antigens on human monocite cell line (THP1-CD14) which express TLR 2, 3, 4, 7, 8 & 9. After screening the interaction of the antigens in conjunction with the corresponding agonist and antagonist of all TLRs we stated that ES antigens

stimulate positively the TLR-4 and TLR-8 and possibly also interact with the TLR-2 and TLR-5. We also stated that only ES antigens in the range of 10-30kDa are involved in the interaction with these TLRs. This fraction includes defined antigens such as GST, Theodoredoxin, Cathepsins, FhSAP2 and FABPs, which are promising vaccine candidates against F. hepatica. Further studies are in progress to elucidate the complete TLRs signaling pathways stimulated by these antigens during the active infection and its influence on the adaptive immune response.

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Leveraging the power of genome scanning to identify the mechanism of resistance in drug-resistant *P. falciparum* parasites evolved in vitro

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Cell-based screens have resulted in the discovery of many antimalarials. However, this screening format carries an inherent disadvantage in that the mechanism of action (MoA) may not be known for the resulting compound hits. NP-1, a compound derived from a hit identified from a cell-based screen, is in late stage preclinical development. Its subnanomolar potency in cellular assays, superior efficacy at low doses in rodent models of malaria, low toxicity in vertebrates and unique chemical structure make this an attractive new antimalarial. Identification of the MoA would help to further characterize this drug. To investigate its possible MoA, drug-resistant parasites were evolved in vitro by continuously culturing in the presence of sub-lethal concentrations of this compound, or to a closely related analog, until resistance emerged. We then used genome scanning to identify the newly acquired lesions in the resistant isolates. All six isolates showed fewer than 50 genomic changes. Most of these differences were in randomly-assorted, subtelomeric genes known to be involved in antigenic variation and which are known to have high rates of change in culture. We observed few changes in the conserved central regions of the genome. However, all six clones contained non-synonymous SNPs in *pfatp4* on chromosome 12, including a copy number variant in one clone. The gene product is characterized as a non-Serca Ca^{2+}-translocating P-type ATPase (PfATP4) and it is believed to contribute to the homeostasis of intracellular concentrations of calcium--a powerfully important second messenger in cell signaling. These data suggest that PfATP4 is either the target or a gene involved in resistance of this new class of antimalarial drugs.

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Parameters determining reproducible evaluation of *In vitro* drug susceptibility of *Leishmania sp.*

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Background Determination of drug susceptibility of clinical strains of *Leishmania sp* is constrained by the variability of results, labor intensity of microscopic evaluation and apparent divergence of in vitro and clinical responses. Identification and control of factors that contribute to variability in the outcome of drug susceptibility testing is needed
Methods We evaluated the influence of temperature during infection, and readout as % cells infected vs intracellular amastigote burden on the evaluation of susceptibility of *Leishmania* to Glucantime® (SbV) and miltefosine (HePC). Susceptibility to miltefosine was also determined for promastigotes. Screening and ED50 analyses were conducted by microscopy and luminometry using *luc* transfected drug susceptible and resistant lines and 28 clinical strains of *L panamensis*.
Results The readout parameter of dose dependent reduction of infection critically affected the accuracy and reproducibility of ED50 determination of both miltefosine and Glucantime®. Concordance between ED50 determined microscopically as % cells infected and parasite burden based on luciferase activity was < 20% whereas ED50 based on microscopically determined parasite burden and luciferase activity was 99.2% concordant. Reduction of infection at the screening dose of both drugs was significantly greater (SbV $p<0.001$; HePC $p=0.018$) when determined as parasite burden (SbV=91.8%; HePC=56.9%) than % infected cells (SbV=77.1%; HePC=19.8%). Incubation temperature above 34°C during infection yielded lower infection and altered cell and amastigote morphology, impairing microscopic evaluation. Both life stages were susceptible to miltefosine at therapeutically achievable concentrations; intracellular amastigotes (ED50=5.3 μM) were more susceptible than promastigotes (ED50=50.2 μM) ($p=0.010$).
Conclusions Parasite burden is a more sensitive and reliable measure of infection than % infected cells. Control of variables that influence readout of infection reduces variability of drug susceptibility of clinical strains.

2990

Evolutionary fate of orthologous genes from Drosophila and Anopheles pericentric heterochromatin

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Pericentric heterochromatin creates an unusual, mostly repressive environment for actively transcribed genes it is harboring. Yet our studies of DNA content of pericentric heterochromatin of European malarial mosquito Anopheles atroparvus revealed the presence of sequences with high similarities to single-copied and vitally important genes from pericentric heterochromatin of Anopheles gambiae. Four of six A. gambiae genes homologous to the Atr2R clones are single-copy and located in heterochromatic regions. All their known Drosophila orthologues are also single-copy and vitally important; however, they reside in euchromatin rather than heterochromatin. Mutations in one of those genes, voltage-gated sodium channel result in knockdown resistance (kdr) to pyrethroids and DDT (Reimer et al 2008). Interestingly, in Drosophila, despite frequent gene transpositions between euchromatin and heterochromatin, some single-copy genes retain their heterochromatic localization, such as the light gene cluster in species of the D. melanogaster subgroup (Yasuhara et al 2005). In this study we report orthologous Anopheles and Drosophila genes that preserve heterochromatic location between these genera and discuss further investigation of their regulation in a repressive environment.

2991

Elucidating the relationship between *Plasmodium falciparum* parasite density and malaria rapid diagnostic test (RDT) band intensity using a novel colorimetric reading device

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Background: Rapid Diagnostic Tests (RDTs) for malaria are lateral flow strips that contain conjugated antibodies, which bind parasite antigens in the blood of malaria positive individuals. The most commonly used antibodies bind Plasmodium genus-specific lactate dehygrogenase (LDH) and *Plasmodium falciparum*-specific histidine rich-protein 2 (HRP2). Upon binding, the antibody-antigen complex yields a visually detectable band. However, detection of these bands is subject to interpretation by the person reading the test. An automated system that quantifies RDT band intensity can offer more objective diagnosis and an examination of the relationship between parasite density and RDT band intensity.
Methods: A colorimetric reader created by Embedded Systems Engineering (ESE) was designed to measure band intensity of Azog brand combo RDTs provided by the Genomix Company. Dilutions of four cultured strains (3D7, W2, DD2, D6) of *Plasmodium falciparum* (Pf) ring stage parasites ranging from 6 p/ul to 100,000 p/ul were run on the RDTs and read by the ESE reader at time points ranging from 15min to 48hrs.
Results: The ESE reader indicates peak stable band intensity for the RDTs occurs 30min to 1hr after running the test. LDH band intensity generally increases with increasing parasite density; however, sensitivity varies by strain. HRP2 band intensity increases with parasite density up to a certain point and then begins to decline; however, this point also varies by strain.
Conclusion: These data suggest some RDTs may require more incubation time than suggested by the manufacturer for optimal reading. The data also contradicts the common belief that RDT band intensity is always positively correlated with parasite density. Rather, the relationship appears to vary for different detection antibodies and strains. Determination of parasite density on the basis of RDT band intensity seems unlikely given these limitations. However, a semi-quantitative approach may be feasible in certain settings if reliable standard curves relating parasite density and band intensity can be established.

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Serological and Molecular Evidence of *Rickettsia massiliae* Infection in Los Angeles, California

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Background: The Los Angeles County Veterinary Public Health Animal Surveillance Program was notified about two ill dogs on one property. They both seroconverted to spotted fever group rickettsiae (SFGR) and had a heavy infestation with the brown dog tick, *Rhipicephalus sanguineus*. Further investigation was conducted because dogs are good sentinels for human Rocky Mountain spotted fever (RMSF) and *Rh. sanguineus* has been associated with several fatal outbreaks of RMSF.
Methods: EDTA whole blood and sera were collected from both case dogs and from two housemate dogs and tested by PCR and microimmunofluorescence assay (IFA) with rickettsial antigens. *Rh. sanguineus* were collected from both the property and directly from the four dogs and tested for SFGR by PCR and sequencing. History and clinical course of canine illnesses were obtained. Dogs

at local shelters were also surveyed for *Rh. sanguineus*.
Results: In spring of 2009, the four dogs were tested and all had high IgG IFA titers to *Rickettsia massiliae* (geometric mean titer, GMT 1:1448), *R. rhipicephali* (GMT1:512), *R. rickettsii* (GMT 1:430) and *Rickettsia* 364D (GMT 1:304). Rickettsiemia was not detected by PCR in these dogs. Of fifty ticks, 32 (64%) were PCR positive for SFGR. Ten to 155 (average 56) *Rh. sanguineus* were collected from the four dogs, and 19 to 80% (average prevalence 34%) of those ticks were PCR positive for SFGR and identified by sequencing as *R. massiliae*. DNA of *R. massiliae* was also detected in *Rh. sanguineus* from dogs presenting at 4 local shelters.
Conclusion: We demonstrated for the first time the presence of *R. massiliae* in *Rh. sanguineus* in California and its association with sick dogs. Although not currently reported from the USA, *R. massiliae* causes an eschar-associated rickettsiosis in humans mostly in the Mediterranean countries, and it also infects dogs. The presence of *R. massiliae* in ticks from California suggests that human cases of *R. massiliae* infection may occur in the region but they are unlikely to be recognized if only cross-reactive serologic diagnostic assays are utilized.

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Malaria parasite infection suppresses mucosal responses to Salmonella in the intestine

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In malaria endemic areas, co-infections represent an important, yet understudied cause of mortality, especially in children. An important co-infection in this context is non-typhoidal Salmonella (NTS). While NTS infections are usually limited to the intestinal tract, underlying malaria infection predisposes children to systemic bacterial spread and lethal NTS septicemia. We have recently developed a mouse model of human co-infection with Plasmodium falciparum and NTS using the rodent malaria pathogen P. yoelii nigeriensis and S. enterica serotype Typhimurium that replicates key clinical features of human co-infections. In this model, underlying infection with P. yoelii suppressed proinflammatory cytokine responses to bacterial pathogen-associated molecular patterns that are critical to keeping bacterial replication at systemic sites in check. Further, neutrophil influx and proinflammatory cytokine responses to NTS are significantly attenuated in intestinal tissue of mice that are co-infected with the P. yoelii. Transcriptional analysis at the genomic level has revealed several gene clusters that are differentially affected during co-infection when compared to single infections. Importantly, mice that were co-infected with P. yoelii demonstrated significantly higher loads of NTS at systemic sites. Based on these results, we hypothesize that malaria parasite infection may comprise the immunological barrier to systemic spread of NTS from the intestine.

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The potential role of heme oxygenase 1 in *P. falciparum* infection

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Work in murine malaria models has demonstrated a protective role for HO-1 in the pathogenesis of severe malaria. To study the role of HO-1 with regard to disease outcome in *P.falciparum* patients, we have enrolled 274 Gambian children with severe malaria (cases) or uncomplicated malaria (controls) into a study for detailed immunological and molecular analysis, using 9 colour flow-cytometry, RT-PCR, HO-1 ELISA, detection of free heme, and Western blot for HO-1 detection in the cytosolic or nuclear compartment.We found that HO-1 is upregulated in acute disease at the mRNA and the protein level in both severe and uncomplicated cases and identified neutrophils as the major source of HO-1. However, on a per cell basis HO-1 was significantly lower in neutrophils during the acute phase, suggesting that HO-1 is released from neutrophils upon activation. In line with this, we detected significantly increased levels of soluble HO-1 in the plasma during the acute disease, which correlates with parasitaemia, HO-1 mRNA levels in whole blood, and IL-10, which is a well known protective factor in malaria infection. We are currently performing more detailed analysis to relate these findings to disease outcome. We will also determine the cellular compartments where HO-1 is found during acute disease. Further, data on the heat-inducibility of HO-1 will be presented.
We hypothesize that malaria-induced neutrophil-derived HO-1 is secreted into the plasma to neutralize free heme.
By translocating into the nucleus it may act as a nuclear transcription factor and/or facilitate the migration of other transcription factors into the nucleus.

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Anopheles gambiae larval antimicrobial responses upon exposure to a subleathal dose of _Bacillus sphaericus_

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Mosquito larval control is one of the measures in integrated vector control against mosquito borne diseases. Being highly effective against mosquito larvae at very low doses and safe to other non-target organisms, *Bacillus sphaericus* (Bs) has been a predominant microbial larvicide employed for mosquito control. However, little is known about how larvae respond to the larvicide. In the present study, we explored the antimicrobial response of larvae exposed to a low dose of Bs. To obtain the lethal concentrations (LCs), the bioassay was conducted using lyophilized Bs 2362 grown in MBS medium and late third instars of *Anopheles gambiae*. The LC10 was 3.05 ng/ml and the LC50 was 13.7 ng/ml. Late third and early fourth instar larvae were treated with Bs at a dose of LC_{10}. The larvae were collected at 3hr, 6hr, 12hr, 24hr, 48hr and 72hr post treatment to make cDNAs. The Real-Time RT-PCR was used to assay the transcript abundance of three antimicrobial genes. The expression of *Cecropin 1* (*CEC1*) showed a two-peak pattern. In comparison to the abundance in the untreated larvae, the *CEC1* expression sharply increased by 6 folds at 3 hr, then subsided to a 2-fold increase around 24hr. The abundance climbed up again by a 7-fold increase at 72hr. The *Defensin4* (*DEF4*) expression went higher progressively from 3 to 72 hr. The *Cathepsin D* (*CathD*) abundance increased by 3 folds at 48hr and went down to the normal level at 72hr. The double-peak pattern of *CEC1* suggested two waves of responses. The gut cell damage caused by Bs toxins may open an access to the hemocoel, leading to a septic infection. The second response of *CEC1*, the progressive upregulation of *DEF4* and the single peak of *CathD* at 48hr may reflect a systemic response to the Bs exposure.

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Development of a Macaque Model for in utero Chikungunya Virus Infection

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus associated with epidemics of debilitating arthralgia in humans. In 2005 a CHIKV genotype from East Africa traversed the Indian Ocean in an epidemic that involved the Comoros, La Reunion, Mauritius and Seychelles Islands and spread to the Indian subcontinent in 2006 with >2 million human disease cases reported. In addition to the magnitude of the outbreak, novel disease syndromes were identified that included neurological manifestations in adults as well as fetal encephalopathy associated with pre-partum neonatal transmission. It remains to be determined whether the novel disease manifestations observed in the current outbreak have been the result of increased virulence or altered tissue tropism of the emergent virus strain or is merely reflective of the size of the outbreak and/or improved case reporting. Although a CHIKV mouse model has recently been developed, non-human primates possess reproductive and immunological systems more reflective of humans, thus allowing for a more accurate representation of human CHIKV tropism and clinical syndromes. In the present study six pregnant rhesus macaques beyond gestational day 120 were inoculated subcutaneously with 1,000 PFU of either an epidemic CHIKV isolate obtained from a viremic human during the recent Indian outbreak or an enzootic Senegal strain from 1983. No significant virological differences were observed between macaques inoculated with either strain. Both groups developed detectable viremias that persisted for 4-5 days with peak viral magnitudes of 5-6 $\log_{10}$ PFU/mL sera observed at approximately 2-3 dpi. Decreased heart rate was identified in all fetuses following inoculation with both strains. Clinical signs of dam and fetus, systemic cytokine and immunological response profiles and histopathology were examined as additional correlates of disease severity. The findings of this study and a description of the implications of this model for investigating the mechanisms responsible for modulation of disease phenotypes will be presented.

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The art of the feasible: assessing the technical, operational, and financial viability of malaria elimination on the islands of Zanzibar

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Introduction. Zanzibar's malaria burden has decreased from hyperendemic levels to <1% prevalence. The Malaria Control Program accordingly finds itself at a crossroads: it can continue its control program indefinitely or attempt to eliminate malaria. To support this decision we evaluated whether and how malaria elimination could be achieved and sustained. **Methods.** Feasibility of elimination was defined along three dimensions: technical, operational, and financial. Technical feasibility of elimination was evaluated using mathematical models that estimated the reductions in transmission achievable with IRS/LLINs. Additionally, a stochastic simulation was used to evaluate the potential for several strategies to maintain elimination despite constant importation of parasites from the mainland. The operational feasibility component of this assessment evaluated whether the interventions needed to achieve and sustain elimination according to the technical models could be implemented given the capacity of the national malaria program and the health system. The financial feasibility component compared the costs of required elimination interventions to those for sustained control. **Results.** Models indicated that elimination is feasible with current tools; local transmission may be interrupted in about a decade if 75% of the population is effectively protected by control measures. Maintaining elimination despite ongoing importation will require detecting a high percentage of imported and secondary cases through passive and active surveillance, with this percentage varying in accordance with importation rates and vector control coverage. Financial benefits will depend upon the extent to which expensive control measures can be scaled back, although the risk of future drug and insecticide resistance increases the benefit of elimination. **Discussion.** Regardless of whether Zanzibar pursues elimination or sustained control, ensuring adequate funding will be essential to avoid resurgence. This assessment provides a framework for other countries contemplating elimination.

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Modeling In-host Dynamics of Malaria with Innate and Adaptive Immunity: Model Calibration for Agent Based Communities

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Individual- or agent-based modeling of malaria infection offers an attractive alternative to the conventional (Ross-Macdonald) or population-based methodology. It allows accommodating heterogeneous 'host/vector/parasite' communities and realistic transmission environment. However it requires more computational resources.
To provide the basis for the establishment of a simulated community of hosts a new, individual based 'parasite - immune effector' model was developed and calibrated using patient data from malaria-therapy studies.
A two step calibration procedure was used to account for a deterministic and a pseudo-random component of malaria therapy patient histories.
The model and calibration procedure presented here, while simplistic, can give reasonable prediction of malaria therapy patient histories, similar in accuracy with more detailed models proposed earlier. By screening a data set of 127 malaria therapy patients we collected a total of 3290 best parameter choices. The resulting parameter set will serve a basis for development of agent-based communities, to study malaria transmission and control in realistic environment with multiple host-parasite-vector strata, and heterogeneous populations.

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Monitoring progress to global malaria eradication: use of placental malaria and infant malaria as tool.

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Introduction: Methods such as ITN use, intermittent preventive treatment, use of ACTs, indoor residual spraying and malaria vaccines are being explored to achieve effective malaria control. There is the need to document current malaria epidemiology in special groups such as pregnant women and their newborns as a way of monitoring the efforts to global malaria eradication.
Methods: A prospective cohort study is being conducted among pregnant women and their infants to determine current malaria epidemiology in central Ghana . Infants have been followed up at least six months for the monthly prevalence of malaria parasitemia. Placental histology was analysed for malaria infection and compared with their infant's malaria in the first 6 months of life.
Results: Data from 102 mother-infant pairs were analyzed. 57%(57/100) of women had placental malaria (active, past, or chronic

infection). The mean age of women with and with and without placental malaria was 26.2yrs(95% CI 24.7 - 28.1) and 28.5 yrs(95% CI 26.7 - 30.3) respectively. Women without education were similar among women with or without placental malaria (43.9% and 48.8% respectively, p=0.62). ITN use among women with or without placenta malaria were similar (49.1%. vrs 41.9%, p=0.47). The proportion of low birth weight was similar among both groups 8.0%(4/46) and 4.9%(2/39); p=0.55. There was no malaria parasitemia in the first two months of life. By 3 months, proportion of malaria parasitemia among infants born to mothers with placental malaria was 4.3% (2/46) compared with infants born to women without placental malaria was 2.6% (1/38); p=0.67.
Conclusion: The conduct of placental histology and monthly infant peripheral blood assessment for malaria is feasible in monitoring the efforts towards malaria eradication. Malaria in the neonatal period is virtually absent and starts appearing by month 3 irrespective of maternal placental malaria status. An extended followup period and a higher number of mother infants pairs will be useful in monitoring the impact of malaria interventions

3001

Antimalarial Activity of Tigecycline, a Novel Glycylcycline Antibiotic.

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Spreading resistance of Plasmodium falciparum to existing drugs and first reports of artemisinin-resistance call for the search for novel antimalarial drugs. Antibiotics with antimalarial activity such as azithromycin, doxycyline, and clindamycin in combination with traditional antimalarial drugs (e.g. quinine, artesunate) are an interesting option for treating multidrug-resistent falciparum malaria.
Tigecycline, a novel glycylcycline antibiotic is a semi-synthetic derivative of minocycline with a broad antibacterial spectrum and a unique and novel mechanism of action in bacteria.
Tigecycline was successfully tested in 66 clinical isolates of Plasmodium falciparum from Bangladesh using the HRP2 in vitro drug susceptibility assay. The 50%, 90% and 99% inhibitory concentrations of tigecycline were 699 (95% CI: 496 to 986), 5,905 nM (4,344 to 8,028) and 12,416.87 (9,481.71 - 16,260.65). Tigecycline shows no activity correlation with traditional antimalarials and has substantial antimalarial activity on its own. The lack of any activity correlation with traditional antimalarial drugs suggests a different mode of action and the absence of potential cross-resistance.
With an IC50 in the nanomolar range and a relatively steep dose-response curve tigecycline shows one of the highest activities of all antibiotics against P. falciparum. In our study tigecycline was up to 6 times more active ($p<0.0001$) against P. falciparum than doxycycline.
Tigecycline showed a significant activity correlation with doxycycline (R=0.51; P=0.003; N=32) but no evidence of a correlation with any of the other tested antimalarials (dihydroartemisinin: R=0.038; P=0.775; N=58, mefloquine: R=-0.159; P=0.234; N=58, quinine: R=0.210; P=0.114; N=58, chloroquine: R=0.198; P=0.135; N=58 and azithromycin: R=0.252; P=0.057; N=58).
We conclude that tigecycline has substantial antimalarial activity on its own and may be a potential candidate for exploring its clinical efficacy in combination with faster acting antimalarials (e.g. artemisinins or quinine) in the parenteral treatment of multidrug-resistant falciparum malaria in seriously ill patients.

3002

Malaria Control in Nigeria - Universal Campaign for use of Long Lasting Insecticidal treated Nets in Anambra State Nigeria. Lessons learnt.

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LONG LASTING INSECTICIDALTREATED NETS (LLIN) UNIVERSAL CAMPAIGN IN ANAMBRA STATE, NIGERIA

Introduction
Malaria continues to be a major public health problem in Nigeria, with children under five years and pregnant women being mostly affected. The use of insecticidal treated nets(LLINs) has been proven to be a very cost effective intervention strategy for the prevention and control of malaria.
The Anambra LLIN Universal Coverage campaign is part of the National Malaria Control program Strategic Plan for Malaria Prevention to deliver 2 LLINs to every household. Anambra served as a pilot State to distribute a total of 1.8 million LLINs to households in all LGAs of the State.
Objective
To distribute 2 LLINs in all households in all the LGAs in Anambra State by 21 July 2009, reduce malaria mortality and morbidity by 50% by the end of 2010 and achieve household coverage of at least 80% by 2010.
Methodology
House hold mobilization and registration/listing, issuing of net cards, community mobilization/demand creation, distribution of nets, complementary use of rapid SMS, in and end process monitoring, daily State & LGA debriefing meetings, waste management and

post campaign follow up process were used.

Results

Out of 1,787,994 nets supplied to the State, 1,613,141(90.2%) were distributed. The total no of Households targeted based on National census figures was 892,262. and 957,039 households were mobilised, given a mobilization rate of 107%.Number of estimated households based on National population estimate was far less than the no of households on ground, suggesting need for enumeration of households before such excercises for accurate planning. Not all families mobilised were given nets because of logistics problems such as difficult terrain. One major gap was non inclusion of Boarding Institutions like Schools and Destitute homes who actually need these nets, moreso when school children are likely to be champions and agents of net use in their homes as net ownership is different from net use. Crowd control was a major issue but was controlled using local vigilante and Law Enforcement agencies. Rumours that LLINs are harmful were contained through massive Radio-TV campaigns and Church announcements.

CONCLUSION

Massive campaigns like this need long term planning involving all tiers of Government and stakeholders, current household enumeration and appropriate logistics.

3003

Health Profile of Children Working as Pesticide Applicators

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Objectives: To examine the impact of pesticide exposure on measures of clinical and biochemical health effects in children and adolescents working as pesticide applicators.
Methods: Male children currently applying pesticides between the ages of 9 and 19 years (n = 50) were recruited for the study. They completed work, health, and exposure questionnaires, medical and neurological screening exams along with specific neurological tests for sensory and motor functions including; cranial nerves, sensory and motor system, and reflexes. Blood samples were collected to measure acetyl cholinesterase, and liver and kidney functions. Children who never worked in agriculture (n = 50), matched on age, education, and socioeconomic level also participated in the study.
Results: More neuromuscular disorders were identified in pesticide applicators than controls. A significant lower level of acetyl cholinesterase was found in the applicator group compared to the controls. There was also a significant difference in hematological, renal and liver indices in the exposed children compared to the control children. Working more days in the current season and also working more years as a pesticide applicator were both associated with an increase in prevalence of neuromuscular abnormalities and significant changes in the biochemical analyses.
Conclusions: We found that children working as pesticide applicators had more neuromuscular disorders, lower acetyl cholinesterase and different hematological, renal and liver indices than control children not working as pesticide applicators. This study replicated the findings of research on adult cotton workers.

3004

Window screening, ceilings and closed eaves as sustainable ways to control malaria in Dar es Salaam, Tanzania.

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Background
Malaria transmission in Africa occurs predominantly inside houses where the primary vectors prefer to feed. Human preference and investment in blocking of specific entry points for mosquitoes into houses was evaluated and compared with known entry point preferences of the mosquitoes themselves.
Methods
Cross-sectional household surveys were conducted in urban Dar es Salaam, Tanzania to estimate usage levels of available options for house proofing against mosquito entry, namely window screens, ceilings and blocking of eaves. These surveys also enabled evaluation of household expenditure on screens and ceilings and the motivation behind their installation.
Results
Over three quarters (82.8%) of the 579 houses surveyed in Dar es Salaam had window screens, while almost half (48.9%) had ceilings. Prevention of mosquito entry was cited as a reason for installation of window screens and ceilings by 91.4% (394/431) and 55.7% (127/228) of respondents, respectively, but prevention of malaria was rarely cited (4.3%, 22/508). The median cost of window screens was between US $ 21 30 while that of ceilings was between US $301 400. The market value of insecticide-treated nets, window screening and ceilings currently in use in the city was estimated as 2, 5 and 42 million US$. More than three quarters of the respondents that lacked them said it was too expensive to install ceilings (82.2%) or window screens (75.5%).
Conclusions

High coverage and spending on screens and ceilings implies that these techniques are highly acceptable and excellent uptake can be achieved in urban settings like Dar es Salaam. Effective models for promotion and subsidization should be developed and evaluated, particularly for installation of ceilings that prevent entry via the eaves, which are the most important entry point for mosquitoes that cause malaria, a variety of neglected tropical diseases and the nuisance which motivates uptake.

3005

Knowledge, Attitude and Practices of Dengue prevention
Jazan, Kingdom of Saudi Arabia (KSA)

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Knowledge, Attitude and Practices of Dengue prevention
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Introduction
Flavivirus Epidemics of a dengue-like disease appeared in the Arabian Peninsula in the late 18th century. Saudi Arabia have conducted a campaign in an attempt to control dengue fever, after 402 residents in Jeddah were found to have the disease.
Objective:
The aim of this study was to assess the knowledge, attitudes, toward prevention of dengue fever and prevention practices among residents of Jazan Province, Kingdom of Saudi Arabia (KSA).
Methods:
A cross-sectional survey was conducted with an interview consisting of 51 items that included Knowledge questions relating to Dengue Fever (DF), attitudes toward prevention, practices of the common preventive measures. The study involved a total of 671 households in four districts in Jazan region . They had been identified through the random sampling using the Primary Health Care (PHCs) using a structured questionnaire.
Results:
It was found that the respondents 60.7% live in urban area, 39.3% rural residents. Twenty per cent were illiterate, 18.6% were university's degree holders. The main source of information about dengue was through TV programs (46%), health care providers (11.0%) and Ministry of Health (MOH) outreach program (2.9%). Very Significant associations were found between respondents' level of education and knowledge ($p=0.000$); Level of education and attitudes toward preventive practices on dengue fever ($p<0.001$). Overall, 45.4% (300) of respondents didn't hear about dengue fever. More than 50% (394) didn't know what to adopt for prevention of dengue fever. In addition, 81.2% of participants prefer to use spry to keep mosquitoes away, while 11.2% use bed nets.
Conclusion:
The results of this study showed that there were significant gaps in the people levels of awareness for the prevention of dengue fever. The importance of health education on DF is the cornerstone to raise their level of knowledge about the way to deal with the disease and how to prevent the disease. Since TV was the important source for delivering dengue information, more TV awareness programs are needed. House visits to demonstrate specific control measures, will play an effective role in dengue prevention.

3006

Anti-malarial activity of the coccidiostat, decoquinate

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Decoquinate (DQ) is an approved anticoccidial drug used in veterinary medicine that has long been used in feed stocks. DQ is structurally similar to WR194905; a compound that was previously shown to have anti-relapse activity in primates.[1] In human and mouse liver microsomal incubation assays DQ exhibits a half life greater than 60 minute. In addition, DQ has shown in vitro blood stage activity against P. falciparum with IC50 values of 175, 6.5, 7.8 9.18 ng/ml against C2b, D6, W2 and C235, respectively. DQ also exhibited activity against P. berghei in an in-vitro liver stage assay with an IC90 of 6.2 ng/ml. In-vivo, DQ exhibits suppressive blood stage activity against P. berghei in mice when administered both oral and SC. In a P. berghei exo-erythrocytic mouse model of malaria DQ completely protected 5/5 mice when administered IP at 40 mg/kg and protected 4/5 mice at 10 mg/kg. Experiments to determine whether DQ possesses anti-relapse activity in primates are currently in progress.
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3007

Nucleosomal changes are associated with transcriptional upregulation of *Plasmodium falciparum* receptor genes in a sialic acid-independent invasion pathway

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A *Plasmodium falciparum* clone Dd2 requiring erythrocyte sialic acid can be switched to a sialic acid-independent progeny clone Dd2NM by growing the clone in neuraminidase-treated erythrocytes. Two contiguous genes in opposite orientations, *RH4* and *PEBL,* are transcriptionally upregulated in Dd2NM, despite the absence of DNA changes in and around the genes. To determine the potential epigenetic modifications around the transcription start site (TSS), the TSS was mapped. The distance between the two 5' TSS of the genes was 170 bp. Nucleosome-scanning and Solexa mapping analyses indicate that the +1 nucleosome downstream of the TSS of the active *RH4* gene in Dd2NM was markedly reduced. Histone H3K4 trimethylation in parallel with acetylations of histone H3 and H4 was positively correlated to the active *RH4* and *PEBL* genes in Dd2NM. Histone H3K9 trimethylation was higher in Dd2 than Dd2NM along the 5-UTRs and coding regions of the *RH4* and *PEBL* genes. Our data indicate that changes of nucleosomes surrounding the TSS play a key role in transcriptional regulation of these *P. falciparum* genes.

3009

Malaria And CD36: Integrins beta1/beta2, and CD13 Stably Associate with CD36 in Mouse Macrophages.

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Innate immune response is essential in controlling early malaria parasite replication and decreasing the risk of progression to severe and fatal disease. We have recently shown that a scavenger receptor, CD36, modulates innate immune response to malaria by regulating cytokine production and parasite clearance. In the present study, we sought to identify signalling mediators downstream of CD36, as they may represent novel therapeutic targets.
Thioglycollate-elicited peritoneal macrophages (PMM) were harvested from the Wild-type (WT) or the CD36-deficient (CD36 KO) mice. Membrane proteins were solubilized, and immunoprecipitation was performed using an anti-CD36 antibody. Immunoprecipitates were resolved and visualized on silver-stained gels, which were compared between the WT lysate and the CD36 KO lysate in order to identify CD36-specific interaction partners. Among the bands that were unique to the WT lysate, three bands corresponding to 160, 100 and 17 kDa were excised, analyzed by mass spectrometry, and subsequently validated by Western blotting. Integrin (INT) beta1, INT beta2, and CD13 (also known as the aminopeptidase N) co-immunoprecipitated with CD36 in the WT lysate, but not in the CD36 KO lysate. Next, we examined whether activating CD36 can regulate the activity of INT beta1. CD36 was cross-linked with a mouse anti-CD36 antibody, which was further clustered by anti-mouse F(ab)2 fragments. Immunofluorescence was performed using an antibody designed to detect specifically the activated form of INT beta1.
Our results indicate that clustering CD36 via antibody-cross-linking may activate INTbeta1. Currently, studies are underway to investigate the functional significance of CD36 activation on integrins by examining the effects of CD36 cross-linking on phagocytic uptake mediated by complement receptor 3, which is composed of alphaM-beta2 integrins. In addition, significance of interaction between CD13 and CD36 will be investigated by using macrophages obtained from CD13-deficient mice.

3010

Impact of Insecticide-Treated Bednets (ITNs) on the frequency of *Plasmodium falciparum* genes associated with resistance to sulfadoxine-pyrimethamine and chloroquine in a malaria holoendemic area of western Kenya

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The use of insecticide-treated bednets (ITNs) has been associated with reduction of malaria transmission in sub-Saharan Africa. However, the relationship between transmission reduction by ITN use and the spread of parasite anti-malarial drug resistance genes remains unclear. We investigated the impact of ITNs on the frequency of *Plasmodium falciparum* genes associated with resistance to the anti-malarial drugs sulfadoxine-pyrimethamine (SP) and chloroquine (CQ) in children less than 5 years old in an ITN trial site in Asembo Bay, western Kenya. During the course of the trial the entomologic inoculation rate was reduced by 90%. We randomly

selected 244 smear-positive blood samples collected just prior to ITN introduction (year 1996) as baseline and 237 samples from five years post ITN intervention (year 2001) for genotyping of the dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) genes associated with SP resistance and the chloroquine resistance transporter (*pfcrt*) and multidrug resistance (*pfmdr-1*) genes linked with CQ resistance. Real-time PCR was used to detect codon mutations at *dhfr-51, 59, 108* and *164*, *dhps -437* and *540*, *pfcrt-76* and *pfmdr1-86*. Overall, the frequency of *dhfr* triple mutations and *dhps* double mutations significantly increased from pre to post-intervention, from 48% to 71% for *dhfr* triple mutations and from 22 % to 73% for *dhps* double mutations. There were no *dhfr*-164 mutant parasites detected in both pre and post-intervention. A greater number of quintuple mutant genotype *of dhfr+dhps* (53%) was found in post- intervention compared to baseline (11%). The frequency of *pfmdr1-86* mutation remained unchanged (87% and 86% for pre and post-intervention respectively); however *pfcrt-76* mutant frequency was lower, borderline significance, in post-intervention (90%) compared to baseline (95%). We also observed a significant decrease in mixed genotypes associated with *dhfr-51, 59, dhps-437, 540,* and *pfmdr1-86* post-intervention. Since the use of both CQ and SP was common in the study area before the ITN trial and SP replaced CQ as the national first line treatment for uncomplicated malaria in Kenya in 1998 but Coartem had not yet been introduced during the ITN trial, our results may reflect a combined effect of transmission reduction and drug pressure of CQ and SP on molecular markers. The extent of the impact of ITNs on markers of malaria drug resistance will require further investigation and monitoring.

3011

A novel CD4+ Effector Memory Subset Protects in Murine Malaria

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As infection with malaria does not result in sterilizing immunity, and protection decays in the absence of exposure, there is much debate about the nature of T cell memory to malaria. Using T cells from the B5 TCR transgenic mouse to study the response of Merozoite Surface Protein-1-specific CD4+ T cells to the blood stages of Plasmodium chabaudi, we observed that memory T cells (CD44hi, IL7Ralpha hi) developed, but with delayed kinetics. MSP-1 specific memory T cells were not seen until day 21 post-infection, in the wake of the prolonged presence of effector cells. Analysis of the composition of the memory population by multi-parameter flow cytometry two months post-infection showed a predominance of effector memory T cells, which included late effector memory cells Tem3 (CD62Llo, CD27-), which are enhanced in chronically infected mice. Chronically stimulated memory T cells (CD44hi, CD25-) produced more IL-2 and IL-10 on re-infection than CD44hi memory cells from mice treated with chloroquine, an anti-malarial drug, to clear the chronic phase. Chronically stimulated memory T cells were also better able to delay appearance of parasitemia in immunocompromised recipients, while reducing the peak and limiting pathology; however, they were less good at helping in final clearance. Surprisingly, treating chronic infection generated a population of memory cells, which expanded better in vitro, and on re-infection in vivo than chronically stimulated cells, but was less able to control peak parasitemia. If late Tem subsets are short-lived, as predicted by their short telomere length, protection offered by chronically-stimulated effector memory T cells, particularly Tem3, may explain the observation that immunity to severe malaria in humans is best acquired and maintained in conditions of repeated exposure.

3012

DESIGNING NEW TOOLS FOR MEASURING THE IMPACT OF ANTIMALARIAL DRUGS ON GAMETOCYTOGENESIS

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Blood-stage gametocytogenesis of the malarial parasite represents an essential step for mosquito transmission of the disease. Consequently, the progression of sexual development has been under intense scrutiny, and there is considerable interest in identifying safe and effective transmission-blocking drugs that would prevent the spread of the disease. Gametocyte clearance is of particular importance in *Plasmodium falciparum* infections, as sexual forms of this parasite can persist in the blood for more than 20 day and as such have a significantly longer lifespan compared to other *Plasmodium* species. During their period of development, *P. falciparum* gametocytes transition through five morphologically defined stage (I-V), which can exhibit distinct drug phenotypes. Moreover, not all antimalarial drugs have gametocytocidal effects, one example being chloroquine, to which mature sexual forms are thought to be less susceptible than asexual blood stage parasites. Others such as sulfadoxine-pyrimethamine, in contrast, appear to stimulate gametocyte production. Finally, worsening resistance to antimalarials renders the search for effective treatments more urgent, in which transmission and more particularly, transmission of resistance should not be overlooked.
The goal of our project is to determine which clinically important antimalarial drugs affect *P. falciparum* gametocyte development, as a means of assessing their ability to inhibit parasite transmission to *Anopheles* mosquitoes. To this end, we have engineered several reporter parasite lines in the gametocyte-producing NF54 background, allowing us to monitor the maturation of sexual forms. Experimentally, a GFP-luciferase fusion driven by promoters specific for distinct stages of gametocytogenesis was integrated at an unmarked *cg6-attB* locus, generated through a first round of genetic manipulation. After stimulating these reporter parasite lines for gametocyte production, we confirmed the gametocyte-specific expression of both reporters in at least 2 of our transgenic lines. In a subsequent step, the effects of various antimalarials on gametocytogenesis are analyzed by monitoring GFP fluorescence and luciferase activity signals.

This work should provide further information on the gametocytocidal effect of current antimalarial treatments as well as offer a new tool for the identification of new compounds affecting parasite transmission.

3013

***Caudal* and *Peptidoglycan Recognition Protein LA* (PGRPLA) in *Anopheles gambiae* anti-*Plasmodium* defense**

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The innate immune system of the African mosquito vector for the human malaria, *Anopheles gambiae* , is able to defend against *Plasmodium* infection mainly via the Toll and Imd (Immune Deficiency) signaling pathways. The mosquito appears to employ its antibacterial defense system against *Plasmodium*, and the presence of bacteria in the midgut, a primary site for *Plasmodium* invasion and development, results in the induction of AMPs (antimicrobial peptides) and other immune-specific genes that act against *Plasmodium* (Dong *et al.,* 2006 & 2009). We are currently investigating the roles of peptidoglycan recognition protein LA (*PGRPLA*) and *Caudal* in *A. gambiae* anti-*Plasmodium* defense. In the *Drosophila* Imd pathway, *PGRPLC* and *PGRPLE* are responsible for Imd activation upon gram-negative bacteria infection. In *A. gambiae*, *PGRPLC* has been indicated as playing central roles in Imd signaling, antibacterial, and anti-*Plasmodium* defense (Meister *et al.,* 2009). We are specifically looking at the intracellular receptor *PGRPLA*, and we have recently observed an increased mortality in *PGRPLA* silenced mosquitoes at ~ 24 h after a *P. falciparum*-infected blood meal. We are further investigating the dynamics of *Plasmodium* infection in *PGRPLA*-KD (knockdown) mosquitoes. Our second aim is to look at *Caudal* as a potential immune factor inanti-*Plasmodium* defense. In *Drosophila*, *Caudal* is a developmental transcription factor as well as an innate immune regulator of AMP gene expression. *Caudal*-KD in *Drosophila* led to AMP over-expression, changes in midgut microbiota composition, midgut cell apoptosis, and mortality (Ryu *et al.,* 2008). Upon silencing of *Caudal* in *A. gambiae*, there is modification of midgut microflora composition as well as limited microflora proliferation. Such preliminary data suggests that *Caudal's* modulation of the midgut microflora may influence anti-*Plasmodium* defense. We are currenty investigating the role of *Caudal* in modulating *Plasmodium* infection.

3014

Usefulness of purified proteins from excreted/secreted trypomastigote antigens from *T. cruzi* (TESA antigens) using affinity chromatography (Concanavalin A) for the diagnosis of Chagas disease.

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Trypomastigote excreted/secreted antigens from *T. cruzi* (TESA antigens) belong to the *T. cruzi* transialidase family responsible for the transfer of exogenous sialic acid to acceptor molecules on the trypomastigote surface. These proteins have been used in different formats for the diagnosis of Chagas disease. The aim of this study was to purify excreted/secreted trypomastigote proteins from *T. cruzi* using affinity chromatography for the diagnosis of Chagas disease. TESA antigens were purified using a Concanavalin A resin and an elution buffer containing a mixture of α-D-mannopyranosyl and α-D-glucopyranosyl. SDS-PAGE was performed to identify the purified bands and a Western blot assay was done to identify the immunogenic bands. SDS page stained with colloidal Comassie permitted the visualization of 3 bands of 220, 170 and 20 kDa while silver staining revealed 5 bands (220, 115 85, 83 and 20 kDa). Western blot using a pool of confirmed seropositive sera for Chagas disease revealed 5 immunogenic bands of 220, 85, 45, 32 and 20 kDa. These data suggest that the purified TESA proteins have potential as a useful new tool for the diagnosis of Chagas disease.

3015

Assessing the impact of delayed density dependence on larval populations of Aedes albopictus

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Aedes albopictus, a species shown to transmit West Nile Virus and Chikungunya, is primarily a container breeding mosquito. The females lay their eggs in artificial containers of water, where the larvae grow until pupation. The potential for disease transmission by Ae. albopictus has increased our need to improve control methods for this species. Most studies focus on the short term effect of a control program, but we also need to understand how the control program will influence future populations. Two parameters we know little about is the impact of density dependence and delayed density dependence in the larval stage. This study was done to understand the impact of delayed density dependence in a natural population in Raleigh, North Carolina. Five gallon buckets were divided in half prior to starting the experiment to allow two treatments, control and experimental, in each bucket. These buckets were then placed at 7

locations throughout the city. The buckets were allowed to collect natural rain water and debris. The buckets were left uncovered to allow the natural mosquitoes to lay eggs and populate the buckets. To create the experimental treatment, eggs and larvae were removed daily, mimicking a larvicidal control method. For the control treatment, eggs were allowed to hatch and pupae were collected as they emerged. After five weeks all larvae were removed from both treatments and the buckets were covered with mesh to prevent egg laying. Equal numbers of first instars were added to each treatment in every bucket. Pupae were collected daily and adults were frozen as they emerged. We then looked at the impact of density on percent pupation and adult body size. A simple sign test indicated that there was an effect of delayed density dependence on pupation. The data is being analyzed using more tests.

3016

Selection of mutations to detect MDR TB in Shanghai, China

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Novel tools are urgently needed for the rapid, reliable detection of multi-drug resistant (MDR) and extremely drug-resistant (XDR) strains of *Mycobacterium tuberculosis*. To develop such tools, we need information about the frequency and distribution of the mycobacterial mutations and genotypes that are associated with phenotypic drug resistance. In a population-based study, we sequenced specific genes of *M. tuberculosis* that were associated with resistance to rifampin and isoniazid in 242 phenotypically MDR isolates and 50 pan-susceptible isolates from tuberculosis (TB) cases in Shanghai, China. We estimated the sensitivity and specificity of the mycobacterial mutations using the results of conventional, culture-based phenotypic drug susceptibility testing as the gold standard. We detected mutations within the 81-bp core region of *rpoB* in 96.3% of phenotypically MDR isolates. Mutations in two structural genes (*katG*, *inhA*) and two regulator regions (the promoter of *mabA-inhA* and the intergenic region of *oxyR-ahpC*) were found in 89.3% of the MDR isolates. In total, 220 (90.9%) of the phenotypic MDR strains had mutations in those regions. Mutations in the *rpoB* core region, *katG*315, and *inhA* -15, were present in 78.1% of the phenotypically MDR strains. Mutations in the intergenic region of *oxyR-ahpC* and *embB*306 can significantly increase the sensitivity of an assay to detect drug resistance. Based on our findings, an approach that prospectively screens for mutations in the *rpoB* core region, *katG* 315, *inhA* -15, the intergenic region of *oxyr-ahpC,* and *embB*306 should detect 90.1% of MDR strains in Shanghai. This study lays the foundation for the development of a rapid, reliable molecular genetic test to detect MDR strains of *M. tuberculosis* in China.

3017

Increased urine pterins in children with uncomplicated falciparum malaria and hyperphenylalaninemia

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Introduction: Patients with falciparum malaria develop reversible hyperphenylalaninemia (HPA) (Lopansri, et al. Infect Immun 74:3355, 2006). HPA is relevant to cerebral malaria since brain aromatic amino acid metabolism is critical for synthesis of biogenic amine neurotransmitters. Phenylalanine (Phe) levels are controlled by substrate-level regulation of Phe hydroxylase (PAH), an enzyme activated by elevated plasma Phe and inhibited by elevated intracellular tetrahydrobiopterin (BH4; PAH's obligatory cofactor). This substrate regulation of PAH tightly controls plasma Phe levels. HPA could result from BH4 deficiency or increased pterin synthesis with elevated intracellular BH4 and inhibition of PAH. **Methods:** We prospectively measured urine pterin metabolites and plasma Phe in 62 healthy controls (HC) and 47 with uncomplicated malaria (UM) (6 months to 6 years old) from outpatient clinics at Amana and Mwananyamala district hospitals in Dar es Salaam, TZ. Plasma amino acids were measured by ion exchange chromatography, and urine pterins by HPLC. **Results:** Children with UM had significant HPA (plasma Phe > 80 uM, $p<0.0001$). The Phe:tyrosine ratio (a sensitive measure of Phe regulation; normal ~1.0;) was elevated (≥1.3) more often in UM (43 of 47) ($p<0.0001$; UM *vs.* HC). Likewise, urine BH4 was significantly higher in UM ($p = 0.017$; UM *vs.* HC). Other urine pterin metabolites (dihydrobiopterin, biopterin, and neopterin), and total biopterins were also significantly higher in UM *vs.* HC participants. **Discussion/conclusions:** Thus, UM is associated with elevated pterin synthesis. This is likely due to inflammatory cytokine-stimulated increases in expression of GTP cyclohydrolase, the rate-limiting enzyme for BH4 *de novo* synthesis, with consequent production of neopterins and biopterins (including BH4). Allosteric inhibition of hepatocyte PAH by rising BH4 concentration follows, leading to disrupted Phe homeostasis. Plasma Phe levels rise as the liver is unable to catabolize the increased Phe flux from accelerated protein turnover in malaria infection. Hence, increased intracellular BH4 likely contributes to HPA observed in UM.

3018

Peak Invasion and Persistence of *Plasmodium falciparum* and *P. berghei* in the Salivary Glands of *Anopheles stephensi*

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In order to utilize human and rodent malarial sporozoites at their highest density in mosquito salivary glands for research and clinical trials, we determined the peak period and duration of gland invasion of various strains of the parasites. Mosquitoes infected with 2 strains of *Plasmodium falciparum* and 3 strains of *P. berghei* were determined for their numbers of sporozoites during days 10-30 after feeding on infected blood. We found that *P. falciparum* wild type invaded mosquito glands at the highest peak during days 14-16 post feed and *Pf.* genetically modified strain during days 12-15. The number of sporozoites decreased dramatically on and after day 26 post feed. For the three strains of *P. berghei,* it took longer for them to invade mosquito glands, the peak period ranged from days 16-24 post feed and the sporozoite numbers decreased dramatically on and after day 28 post feed.

3019

Culex erraticus host preference in Sonso Lagoon, Colombia - A generalist and a specialist?

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The host preference of mosquitoes influences enzootic arboviral cycling. Specialists can transmit viruses within a population of the same species, while generalists can bridge infections from maintenance reservoirs to incidental hosts. Arboviruses, such as Eastern and Venezuelan equine encephalitis, St Louis encephalitis and West Nile virus, are amplified in reservoir populations and transmitted to susceptible hosts by mosquitoes that lack host seeking fidelity. Culex erraticus is a competent vector of the first two viruses and a suspected vector of the latter two. Previous blood meal analyses show Cx. erraticus to be a generalist. This study aimed to elucidate the host preference of Cx. erraticus in Sonso Lagoon, a protected wetland in the inter-Andean Cauca Valley in Colombia. Over the course of 5 weeks, from July to August 2008, mosquitoes were collected in resting boxes and birds were counted using point transects. Birds were inventoried visually and by song. DNA was extracted from a total of 300 blood fed Cx. erraticus, 60 from each week. A PCR was run using primers specific to the vertebrate cytochrome oxidase I or cytochrome b gene, results were visualized on an agarose gel and PCR products were purified. These were sequenced and similarity was compared using the NCBI and Barcoding of Life databases. Products were successfully sequenced for 66% (200/300) of samples. Of these, birds composed 72.5% of the blood meals, while mammals were 21% and reptiles were 6.5%. Wading birds (Order: Ciconiiformes) composed 33.2% of all bird counts, but 47% of positive blood meals. Limpkins (Aramus guarauna) were only 2.4% of all birds counted, but comprised 25% of blood fed Cx. erraticus. Also interesting was the absence of blood meals from song birds (Order: Passeriformes), hawks (Order: Falconiformes), and parrots (Order: Psittaciformes), which were 17%, 9.5%, and 7.6% of the total birds present in counts, respectively. These results demonstrate a generalist approach in terms of broad host preference, but also a specialist affinity for particular types of wading birds.

3020

Wolbachia, the Achille's heel of filarial diseases: a drug target from Drosophila to Nematodes.

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Filariasis regroups diseases due to parasitic nematodes transmitted mainly by blood-sucking insects, and infecting more than 120 million people with over 1 billion at risk in tropical regions. Nematodes invade mainly the lymphatic system -i.e. Brugia malayi-, or subcutaneous layer of the skin -i.e. Oncocercha volvulus-, causing ultimately Elephantiasis or River Blindness respectively. The recent discovery that these parasitic worms rely on the bacterial endosymbiont Wolbachia for viability has opened up new opportunities for combating nematode-based diseases. Toward this goal, we have undertaken a detailed cytological study of Wolbachia localization in Brugia malayi adult tissues and embryos by immunofluorescence techniques that we will report.
In parallel we are conducting a drug screening on Wolbachia-infected Drosophila cell cultures. We found a dozen of drug candidates decreasing significantly the bacterial titer in cell cultures in a library of about 2,000 compounds. These potential candidates were retested in a secondary screen in Brugia malayi. Motility tests have shown 5 of these drugs to act faster than the currently used Ivermectin or Doxycyclin, giving serious hopes for more potent treatments in the near future.

3021

Seroprevalence of Toxoplasma gondii in goats from Southwestern Mississippi

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Toxoplasmosis is a disease caused by the intracellular protozoan parasite, Toxoplasma gondii, which is believed to be cosmopolitan in the human population. T. gondii causes still birth, abortion; and neurological problems in human beings. It is non-host specific. Although it is the parasite of cats, it occurs in marine and terrestrial animals, especially goats. With the consumption and production of goat products increasing at a high rate in the United States, toxoplasmosis has become a major health concern. This study was conducted to contribute to knowledge on the prevalence of T. gondii antibodies in goats in Mississippi. During the period of February to April, 2009, a total of 100 serum samples were collected from goats in five Mississippi counties located in the southwestern part of Mississippi, namely, Adams (42), Franklin (12), Lincoln (22), Stone (6), and Wilkinson (12). The samples were tested in three serial dilutions of 1:25, 1:50, and 1:500 using the modified agglutination test (MAT) method. A titer of 1:25 was considered to be seropositive. This study indicated that 24 (24%) of 100 goats were seropositive for Toxoplasma antibodies at 1:25 titer and, 10 (10%) at 1:50 titer and 1 (1.00%) at 1:500. A total of 24 (24%) out of 100 samples tested was positive. This study covers some counties not previously surveyed.

3022

Dengue Seroprevalence in a Campus Community in St. Kitts and Nevis

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Introduction: Dengue outbreaks occur periodically in St. Kitts and Nevis. The objective of this study was to determine the seroprevalence of dengue among members of the Ross University School of Veterinary Medicine (RUSVM) campus community.

Methods: The RUSVM is an American veterinary school located in St. Kitts. The campus community is comprised of individuals from both dengue-endemic and non-endemic countries; most students and faculty are from North America while most of the staff are from St. Kitts and Nevis. A random sample of students, faculty, and staff was selected, contacted, and offered admission into the study. Those who provided informed consent were sent an electronic survey with questions about household characteristics and individual behaviors. They were then asked to have 5cc of blood drawn for dengue testing. Laboratory testing was performed using an IgG ELISA, and the seroprevalence of dengue was determined. Crude and adjusted associations between behavioral characteristics and seropositivity were assessed using logistic regression.
Results: There were 135 persons who agreed to participate (acceptance rate of 56%), 115 of whom provided blood samples. The median age of the participants was 26.5 years (range: 21-71 years), and most (67%) were female. 70% of the participants were students, 15% were faculty, and 16% were staff; and their median duration of residence in St. Kitts was 1.6, 6.5 and 30.0 years respectively ($p<0.001$). The overall seroprevalence was 42%, and the seroprevalence by occupation was 29% in students, 53% in faculty, and 100% in staff. Duration of residence in St. Kitts was significantly associated with seropositivity on multiple logistic regression (p-value = 0.004).
Conclusion: After a substantial increase in reported dengue cases in St. Kitts and Nevis in 2008, 29% of RUSVM students were seropositive. All staff participants were seropositive, while faculty members were more likely to be seropositive with longer duration of residence.

3023

Comparative clinical genomics of artesunate-resistant P. falciparum imported from West Africa

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Background: With Artemisinin derivatives now available for treatment of imported malaria in North America, surveillance of artemisinin resistance is essential in returning travelers. We demonstrate that artesunate resistant clinical isolates do occur in returning travelers from Africa and were amenable to clincally informative comparative genomics.
Methods: P. falciparum clinical isolates (n=24) from returning travelers to Africa during 2008-2009 were cultured and then then tested for drug susceptibility using the SYBR green assay. DNA was extracted from isolates and 3000 SNPs examined by DNA microarray. Previously described SNPs (pfmdr1 and pfATPase) and gene copy number (pfmdr1) were interrogated using pyrosequencing and RT qPCR. Maximum parsimony phylogenetic sequence analysis was carried using Bionumerics.
Results: Drug susceptibility confirmed the presence of elevated IC50 to artesunate in 2 isolates from West Africa (20.13 nM, 16.17 nM) compared to other clinical isolates (mean 9.29 nM). Pyrosequencing revealed that both isolates with resistance to IC50 had the following haplotypes for Pfmdr1 (N86, F184, S1034, N1042, D1246) and PfATPase (E623, N769). The only unique residue for the

two artesunate resistant isolates was N86 of pfmdr1. Elevated pfmdr1 relative gene copy number (1.8) was seen in 1 of the 2 artesunate resistant but not susceptible isolates. The DNA SNP microarray revealed that the 2 artesunate resistant isolates had several genes (n=9) with unique SNPs not present in susceptible isolates. GO function for the 9 unique genes were transport (n=2 [incl pfmdr1]), phosphatidyl synthesis (n=1), tRNA processing (n=1), redox (n=1), phosphorylation (n=1), and unknown function (n=3). Maximum parsimony SNP-based phylogenetic analysis demonstrated a clade of West African isolates to which the artesuante-resistant isolates belong.
Conclusions: We demonstrate that clinical isolates from returning travelers to West Africa have increased inhibitory concentrations to artesunate. This has important clinical treatment implications with artemisinin derivatives avilable in North America. Molecular analysis identified changes in copy number and SNPs in pfmdr1 which correlate with artesunate resistance. Other correlative artesunate resistance genes were also identified using DNA microarray. A clade from West Africa based on DNA microarray SNPs may be an emerging source of artemisinin resistance.

3024

Specific and bystander memory B cell and antibody responses to *Plasmodium falciparum* malaria

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In contrast to the host immune response to many other pathogens, immunity to *Plasmodium falciparum (Pf)* malaria is slow to develop and relatively short-lived. As antibody responses are known to be critical to blood stage immunity, we conducted a year-long prospective study of 185 children and adults in Mali to understand the basis of what is generally presumed to be a suboptimal B cell response to malaria We found that the memory B cells to the blood stage antigens AMA1 and MSP1 are acquired inefficiently despite repeated exposure to the *Pf* parasite, but with infection, their kinetics were similar to that seen with memory T cell response to repeated vaccination-expanding rapidly after re-exposure to antigen, and then contracting to a point slightly higher than pre-infection levels. By FACS analysis we found that a functionally and phenotypically distinct population of $FCRL4^{+}$ hypo-responsive memory B cells was expanded in this *Pf*-exposed population, similar to the 'exhausted' memory B cells observed in untreated HIV-infected individuals, suggesting that *Pf*-associated premature exhaustion of B cells may contribute to the inefficiently acquired and short lived antibody responses against *Pf*. Interestingly, we also observed a modest but statistically significant increase in tetanus-specific MBC but not tetanus Ab two weeks after episodes of acute malaria, providing evidence in support of the notion that memory B cells and long-lived plasma cells are independently regulated, and also calling into question the long-standing hypothesis that *Pf* induces polyclonal differentiation of memory B cells into antibody-secreting cells. Efforts to further understand the cellular and molecular basis of acquired humoral immunity to malaria could inform malaria vaccine design and help determine the optimal timing of booster vaccinations.

3025

Investigation of gene expression patterns of putative antioxidant genes in P. berghei mosquito stages using real-time PCR

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The malaria parasite Plasmodium is transmitted between humans by Anopheles mosquitoes. Within the mosquito, the parasite undergoes a series of fundamental metabolic transformations in order to survive, complete its sexual cycle, and secure transmission to the next host. Prime challenges the parasite faces in the mosquito are reactive oxygen (ROS) and reactive nitrogen species (RNS) which originate from the digestive midgut environment, the insect's immune response as well as the parasites' own metabolism. We therefore hypothesize that Plasmodium absolutely depends on its antioxidant defense systems to survive in the mosquito. However, more than 20 putative antioxidant genes have thus far been described/predicted for Plasmodium and characterizations are mostly restricted to the disease causing blood stages. Using real time PCR we followed the expression of selected antioxidant genes during the first 24 hrs in the mosquito midgut. We investigated whether gene expression is altered in peroxiredoxin KO parasite strains. Lastly we tracked gene expression in wild type parasites from an ookinete culture.

3026

A High-resolution Linkage Map and Estimation of Recombination Hotspots for *Plasmodium falciparum*

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The human malaria parasite *Plasmodium falciparum* relies on its highly diverse genome and its ability to quickly modify its genome to evade host immunity and evolve resistance to antimalarial drugs. High rates of mutation and recombination frequency can play an

important role in its response to these selection pressures. Here we used a high-density tiling array with 2.5 millions probes to estimate frequencies of nucleotide substitution and genetic recombination among 33 progeny of a *P. falciparum* genetic cross (7G8 x GB4). We detected 2254 segregating multiple single-feature polymorphisms (mSFPs) and 632 crossover events among the progeny and constructed a genetic linkage map with 759 informative markers. Comparison of the linkage map with the physical chromosome lengths revealed a high frequency of recombination in the malaria genome. We also estimated a spontaneous mutation rate that is approximately10 fold higher than that found in humans. These results show that *P. falciparum* genome is highly recombinogenic with a high error rate during DNA replication, providing the genetic basis for parasite adaptability to the host immune response and drug pressures.

3028

Spatial stability of *Aedes aegypti* populations

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Spatial stability of adult *Aedes aegypti* exists if its spatial dispersal pattern repeats itself in time, making it predictable. The importance of detecting spatial stability in a vector population is the possibility of applying targeted vector control measures at those places where the mosquito is more abundant. Previous studies on the spatial dispersal of *Ae. aegypti* in urban areas have shown the highly aggregated nature of *Ae. aegypti* pupae and adults. However, spatial autocorrelation of mosquitoes or dengue cases have been shown to disappear beyond 30m. Also, it has usually been difficult to predict where the main clusters of mosquitoes are located within a human community. We studied the spatial dispersal of adult *Ae. aegypti* in two neighborhoods in the city of San Juan, Puerto Rico, using BG-Sentinel traps that were spaced 100m apart. Sampling was conducted for four consecutive days, every three weeks from November 2007 to December 2008.No significant spatial autocorrelations were detected in the number of *Ae. aegypti* females in any neighborhood for most of the sampling instances throughout the study. There was substantial spatial stability as reflected by the low variability in the numbers of mosquitoes captured per trap from one sampling instance to the next. Spatial stability disappeared and significant increases in the adult *Ae. aegypti* population occurred after heavy rains, possibly reflecting increased and generalized recruitment of rain-filled containers. These results have important implications for *Ae. aegypti* and dengue control at the neighborhood level and highlight the importance of spatial and temporal scales in vector studies.

3029

Broadly strain-transcendent epitopes targeted within VAR2CSA-DBL3 and VAR2CSA-DBL5 after rat and rabbit immunizations

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Pregnancy associated malaria is a severe clinical syndrome associated with sequestration of *Plasmodium falciparum*-infected erythrocytes (IEs) in the placenta. Placental binding is mediated by VAR2CSA, a large and polymorphic protein that contains six Duffy binding-like (DBL) domains. To better understand if conserved regions in VAR2CSA can be targeted by antibodies we developed a panel of seven CSA-binding parasites from diverse geographic origins. Overall, no two parasites in the panel expressed the same VAR2CSA sequences and individual DBL domains averaged between a low of ~61% amino acid identity (DBL6) to a high of ~88% amino acid identity (DBL4). However, there was extensive overlap in polymorphism between globally dispersed parasite lines due to *var2csa* gene moisaicism. To investigate if VAR2CSA immunogens can elicit cross-reactive antibodies against parasites in the panel, we expressed the six individual DBL domains from VAR2CSA as recombinant proteins in *Pichia pastoris* and immunized rats and rabbits. Whereas antibodies against most of the VAR2CSA recombinant proteins are able to recognize the homologous CSA-binding parasite line, and have limited or partial cross-reactivity on heterologous parasite lines, the anti-DBL3 and anti-DBL5 sera have exceptional breadth and are able to recognize most of the parasites in the panel. Even though it is still unclear whether adhesion blocking epitopes are conserved, this study demonstrates highly strain-transcendent epitopes in native VAR2CSA protein which could be targeted by DBL3 or DBL5 vaccination and may have application for pregnancy malaria vaccine development.

3031

EVALUATION OF HEALTH-FACILITY BASED VERSUS HOUSEHOLD SURVEYS FOR MALARIA SURVEILLANCE IN TANZANIA: 2002, 2004, 2006

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The scale-up of malaria control efforts in many endemic countries has intensified the need for reliable and timely information for program management. Household surveys (HHS), while an established method for malaria surveillance, are resource intensive, and performed intermittently. Health facility surveys (HFS) could provide ongoing surveillance, as data collection could be efficiently incorporated into existing operations. However, there is a gap in understanding how HHS and HFS data compare. We evaluated malaria indicators (fever and parasitemia) from HHS and HFS performed in two adjacent rural sites in Tanzania biennially from 2002 to 2006.
In the HFS, four facilities in each site were selected purposively to reflect geographic and administrative diversity of the site. Patients of all ages presenting for sick visits were recruited. Fever, clinical diagnoses, treatments, and blood film for malaria were obtained from all subjects. HHS were conducted on a simple random sample of census enumerated households in the catchment areas of the same health facilities. Surveyors recorded history of fever, and collected blood samples for parasitemia. We compared prevalence of self-reported fever and parasitemia between HFS and HHS.
The mean age in years was 15.4 for HFS and 23.5 for HHS. Prevalence of self-reported fever was higher among persons at health facilities than persons in households. Parasitemia prevalence did not differ substantially between HFS and HHS in 2002 (29% vs 24%), 2004 (26% vs 21%), 2006 (10% vs 15%) and over all years 2002-2006, in unadjusted analysis (p=0.06) and after adjusting for known confounders of parasitemia (age, year, and site; p=0.06).
Parasitemia prevalence was similar in both HFS and HHS. Not surprisingly, fever was more common in subjects at health facilities. These findings should be interpreted cautiously, but suggest that prevalence of certain malaria indicators observed in HFS match those observed in HHS in these communities.

3032

Antibodies enhance infection of LSECs in a model of ADE-induced severe dengue disease

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The disease caused by dengue virus (DENV) ranges from dengue fever (DF), a self-limited febrile illness, to the potentially lethal dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS). Epidemiological studies suggest that DHF/DSS usually occurs in patients who, prior to infection, have acquired DENV-reactive antibody, either from a previous infection with a heterologous dengue serotype or, in the case of infants, passively from an immune mother. Therefore, it has been hypothesized that subneutralizing levels of DENV-specific antibodies exacerbate disease, a phenomenon termed antibody-dependent enhancement of infection (ADE). To date, the mechanism of ADE and its contribution to pathology remain elusive, as ADE has never been demonstrated in vivo. In addition, the cell population supporting ADE in vivo has not been characterized. Here, we demonstrate that the presence of anti-DENV antibodies in DENV-infected mice can be sufficient to induce severe disease resembling human DHF/DSS via massive infection of liver sinusoidal endothelial cells (LSECs). Our results contrast with previous studies showing that macrophages, dendritic cells and monocytes are subject to ADE in vitro, and this is possibly due to differences in cell behaviour in vivo versus in vitro. Beyond dengue disease, our findings suggest that suboptimal humoral responses may, under some circumstances, have pathological consequences.

3033

Controlling TB in Nigeria using the Community TB care strategy. Lessons from Community Townhall Dialogue.

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Perspectives of Controling TB in Nigeria using the Community TB Care Strategy
Introduction
Tuberculosis remains a serious public health problem in Nigeria. The magnitude has expanded as a result of HIV/AIDS TB co-infection. One of the new strategies to combat TB scourge is the Community TB Care Dialogue program which makes the community see TB control activities as not Government or Donor Agency concern, but principally the community's concern. Thus the community shows ownership of TB control activities.
Objectives
The objective is to ascertain the Community perspective of TB, improve access to TB Care, empower TB patients and the community through health education and encourage community ownership of TB programs.
Methodology
Seventy two participants were purposively recruited from stakeholders who comprised Community leaders, Religious Leaders, TB patients, Community Development committtees, Civil Society Organisations(CSO), Community Based Organisations(CBO) and Health Workers from all Local Government Areas in Anambra State. The participants were randomly assigned into three groups for the purposes of generating community perspectives of TB - cause(s), symptoms and signs and treatment preferences, quality of available services, stigma and discrimination, barriers to TB services and prevention.
Result
Participants in all the three groups were able to identify the signs and symptoms of TB, but not all knew the bacterial cause. All agreed that TB was a major problem in their community. A large number believed TB could be got from witchcraft. They identified stigma,

poverty and poor attitude of health workers as barriers to seeking health services. Though services were supposed to be free, there were "under the table" collections from health workers and occassionally stock out of reagents and drugs. Participants suggested family care/supervision of Observed Treatment Short course (DOTS) by family members, to minimise travelling cost to health facilities for observation. Communities observed that the long treatment for TB result in noncomplaince and drop outs, and therefore needs home visitation and encouragement by health workers. Participants agreed that community partnership and ownership will reduce Staff poor attitude and improve patronage of health facilities.

Conclusion

Community TB Care Strategy has the potentials to improve TB care services in the community and enhance community ownership of programs.

3034

Expression Patterns of Insulin-Like-Peptides in *Anopheles stephensi*

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Peptides of the insulin superfamily share similar structural motifs across widely divergent tax and regulate a multitude of biological processes, including host response and immunity to infection. Insulin signaling is similarly highly conserved, with recognizable canonical signaling pathway elements in organisms from nematodes through mammals. Recent data show that these signaling pathways can regulate growth, reproduction, innate immunity, and lifespan in *Anopheles stephensi*, a major vector of human malaria in India and Asia. The development and successful transmission of malaria parasites is dependent on the physiological interaction between a mammalian host and a mosquito vector. During feeding and digestion, human insulin in ingested blood activates mosquito insulin signaling pathways. We hypothesize that this signaling - in a manner analogous to that observed in mammals - results in feed-forward signaling that is amplified by the release of endogenous insulin-like-peptides (ILPs). That is, we predict that mosquito ILPs are released and may act in concert with ingested insulin to amplify insulin-dependent responses throughout the body. To test this hypothesis, we developed quantitative real time PCR (qRT-PCR) assays to detect and quantify expression of five endogenous *A. stephensi* ILPs. These assays were used to analyze tissue- and age-specific ILP expression as well as expression of the ILPs in response to physiologically distinct feeding regimes. These regimes included starvation, a phenomenon that likely occurs under natural conditions; sugar feeding, which is manifested as nectar feeding in nature; and blood feeding. Striking differences were noted in the transition to sugar feeding and to blood feeding. These responses were accentuated by the degree of previous starvation, suggesting that finely tuned patterns of ILP activity regulate the switch between sugar gluttony and hematophagy, a phenomenon that could also influence the host response to malaria parasite infection.

3035

UNDERSTANDING UNCERTAINTIES IN THE PREDICTIONS OF A SPATIAL MOSQUITO POPULATION MODEL OF AEDES AEGYPTI

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Aedes agegypti is one of the most important disease vectors in the world. The development of spatial mosquito models for A. agegypti makes it very promising for model-based pest control and risk assessments. One key challenge of that is to understand the reliability of population dynamics predicted from those models, which can be measured by the uncertainties in model predictions. In this study, we quantify the uncertainties in a spatial model of mosquito population (SKEETER-BUSTER) in its application to the Iquitos city within Amazon forest in Peru. Uncertainties in the model predictions are resulted from 69 parameters accounting for mosquito survival, development, fecundity, environmental thresholds, and spatial dispersals. In this study, we employ an advanced uncertainty and sensitivity analysis technique, the improved Fourier Amplitude Sensitivity Test (FAST), which can incorporate correlations among parameters, provide standard errors for estimated sensitivity indices, and can be applied for complex models with nonlinear and non-monotonic structures. Our results show that uncertainties resulted from biological model parameters in the SKEETER-BUSTER model do not exponentially increase through time. This suggests that SKEETER-BUSTER model can provide reasonable predictions of long-term mosquito population dynamics given good estimations of food inputs and biological parameters. The most important parameters contributing to the uncertainties in population dynamics include the survival rates for female adults and larvae, and coefficient of metabolic weight loss for larvae. The stochastic uncertainty is relatively low for the overall population dynamics but relatively high for spatial population dynamics. Our analysis is the first systematic analysis to examine the uncertainties in mosquito population predictions, which can provide a fundamental basis for the applications of mosquito population models toward a model-based population management and a more efficient disease control.

3036

Identification and characterization of the African malaria mosquito PKC gene family

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Of the 400 known species of anopheline mosquitoes, approximately 40 species are important vectors of malaria parasites. Further, it has been proposed that most mosquitoes are naturally resistant to infection with *Plasmodium* spp. and that genetic mutations result in susceptibility and successful parasite transmission. Therefore, the identities of the genes harboring these mutations will provide insights into the mechanisms involved in vector competence. The highly conserved protein kinase C (PKC) gene family has been shown to regulate wide-ranging immune functions in a variety of vertebrate and invertebrate species. Based on these observations, we sought to identify and characterize all of the PKC isoforms encoded within the genome of the African malaria mosquito *Anopheles gambiae* as a prelude to functional studies. A total of 13 PKC isoforms are known from mammals and six are known from *Drosophila melanogaster*. However, prior to our studies, only two PKC-encoding genes had been identified in the genome of *A. gambiae*. Using Hidden Markov Model (HMM) searches of the translated reading frames of the unannotated *A. gambiae* genome sequence, we confirmed the identity of the two previously annotated PKC-encoding genes, identified an additional three PKC-encoding genes and a gene encoding a PKC-related kinase 2 (PKN2) ortholog. Subsequently, we identified conserved domains, putative translational start sites, and phosphorylation sites required for catalytic function of the predicted proteins using ClustalX and manual alignments of transcriptionally validated orthologous sequences. Expression data for of all but one of these PKC-encoding genes have been deposited in publically available databases. Preliminary phylogenetic analyses were performed using PAUP* 4.0. With this new knowledge of the PKC gene family in *A. gambiae*, we can now begin to characterize the functions of these proteins in host physiology.

3037

Identifying Target Areas of Health Education and Intervention to Improve the Effectiveness of an Integrated Management of Childhood Illness-Based Child Survival Program in Northern Tanzania

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Child survival programs implemented cooperatively by local governmental health agencies and NGOs play a pivotal role in improving linkages between health systems and communities; increasing quality education, care and referrals from community-based providers; and promoting essential hygienic household practices for child and maternal health. An assessment of knowledge, attitudes and practices around maternal and newborn care and childhood illness management was conducted as a part of the mid-term evaluation of a USAID-funded, five-year Child Survival program implemented by Minnesota International Health Volunteers. Interviews with mothers of children under 5 (n=30) were carried out using convenience sampling methods in 8 villages of Karatu District, Arusha Region, Tanzania. This work highlighted the need for more aggressive promotion of exclusive breastfeeding (EBF), as only 47% of mothers interviewed believed that breast milk alone was enough for infants younger than 6 months. While the average age for introducing complementary foods was 5.7 months, the majority of infants under 6 months were given porridge, cow's milk and water also. Mothers reported stopping EBF because of illness, pregnancy and the need to resume work. Although knowledge of and access to preferred family planning (FP) methods was relatively high, with 87% of 30 respondents reporting at least one benefit of FP and 63% reporting access to their preferred method of FP, the use of these methods was limited by perceived health risks associated with FP and uncooperative partners. This work demonstrates the importance of a multidimensional approach to preventing childhood illness that includes methods to improve maternal health and empowers women to access and utilize the resources they need to take care of themselves and their children.

3038

Probing the expression and function of two excretory/secretory antigens in different life stages of Fasciola hepatica

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Fasciola hepatica is an important disease of livestock and an emerging human pathogen. Growing resistance to the drug of choice, Triclabendazole, is compromising control options and underscores the need to discover novel chemo- and/or immunotherapeutics. The excretory/secretory (ES) products produced by *F. hepatica* are key players in the host-parasite interaction and offer appealing targets for chemo/immunotherapy. We have identified two ES antigens in adult *F. hepatica* with potential roles in nutrient acquisition: (i) FhFrr, a Ferritin-like protein that is involved in iron (Fe) metabolism; and, (ii) FhSAP2, a member of the Saposin-like protein family (SAPLIP) that has been shown to have lytic activity on human peripheral blood mononuclear cells and erythrocytes. However, the

function of both antigens during the early stages of infection remains unknown. RNA interference (RNAi) provides a tool with which to probe gene function / validate candidate drug/vaccine targets, and has been successfully employed to silence genes in the infective stage (newly excysted juvenile, NEJ) of *F. hepatica*. Using quantitative (q)PCR we demonstrate *FhFrr*- and *FhSAP2*-expression in NEJs; immunocytochemical studies using a rFhSAP2-antiserum are in progress. To probe the function of FhFrr and FhSAP2 in juvenile fluke, we exposed NEJs to double stranded RNA (dsRNA, 100 ng/ul). Preliminary phenotype observations revealed that >70% NEJs exposed to dsRNA-*FhSAP2* exhibited abnormal locomotion compared to control worms; qPCR confirmation of gene silencing is ongoing. *FhFrr*- and *FhSAP2*-RNAi was extended to adults and preliminary results obtained by qPCR revealed inconsistent fold reductions in the levels of *FhSAP2* transcripts between worms. To facilitate adult RNAi future work will involve varying experimental parameters including dsRNA concentration/exposure time and dsRNA design. These preliminary data suggest that RNAi will facilitate functional studies on FhFrr and FhSAP2 in juvenile and adult *Fasciola* and highlight FhSAP2 and FhFrr as potential vaccine/drug targets.

3040

Arboviruses and relative Diseases in China

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Arboviruses are those viruses that can be transmitted by blood sucking insects such as mosquitoes, midges, ticks. They can induce diseases in biting to human or animals. Arboviruses are regarded as viruses causing communicable diseases common to human being and animals. Today, such as Dengue fever, West Nile virus disease, and Rift valley fever still outbreak in the worldwide. Study of arboviruses not only becomes the important topics of virology, but also appears a social problem directly related with the public health. In world, 539 species of arbovirus have been registered in the WHO Center of Arbovirus in USA in 1999. More than 100 viruses cause diseases in human beings. For a long time, the four species of arboviruses and relative disease have been identified in China, Japanese Encephalitis (JE), Dengue Fever (DEN, 1-4 serotype), Tick-born Encephalitis (TBE) and Crimia-Congo Heamarrhagic Fever (XHFV). Over the past years, an investigation on arbovirus has been carried out in China, across 20 provinces of the country. To learn more about arboviruses or viruses spread by insects, 200,000 insect samples have been collected. More than 300 strains of arboviruses have already been isolated from the samples, such as JEVs, alphavirus, Bunyavirus, dsRNA virus and DNA virus.

3041

Intrinsic and Extrinsic Influences on the Probability of Emergence of an RNA Viral Pathogen.

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The potential emergence of RNA viral pathogens remains a consistent threat to Homo sapiens, demonstrated by the last decade's multiple emergence events. The source of this threat rests in the unpredictability through which viruses emerge and cause epidemics, this unpredictability related to gaps in our understanding of viral pathogen biology and ecology: Are their biological or epidemiological characteristics of a viral pathogen that we can use to determine how probable it is to emerge? How does the evolutionary or ecological history of an RNA virus impact its ability to emerge? To address these questions, I combine mathematical modeling with empirical results towards understanding the qualitative and quantitative contributions of important RNA virus characteristics to the probability of emergence. I find that depending on the standard route of transmission, different RNA virus characteristics, such as generalism and extracellular survivorship, can powerfully influence the probability of emergence of a RNA viral pathogen.

3042

ASYMPTOMATIC DENGUE INFECTIONS IN A COHORT OF FACTORY WORKERS

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Infection with dengue virus may result in a broad spectrum of clinical manifestations including subclinical infections, dengue fever, dengue hemorrhagic fever, or dengue shock syndrome. In order to better understand the pathogenesis and epidemiology of dengue, we

followed a cohort of 2,726 factory workers in Bandung, Indonesia for evidence of dengue infection. The factory workers were instructed to come to the factory clinic for a medical examination and blood draw to test for dengue if they did not feel well. Additionally, all cohort members had their blood drawn at sero-surveys conducted every four months.
In order to screen for asymptomatic dengue infections in this cohort, the set of sero-survey specimens collected over 18 months (2007 and 2008) from a random sample of 80 cohort members was analyzed using a commercial anti-dengue IgG ELISA kit. Suspected asymptomatic infections were identified by consecutive sero-survey samples having an increase in anti-dengue IgG above an established criteria. Forty-three suspected asymptomatic dengue infections were identified in this subset of the cohort, consisting of 4 primary and 39 secondary infections. During the same time period, 43 symptomatic dengue infections (28 dengue fever, 6 dengue hemorrhagic fever grade 1 and 9 dengue hemorrhagic fever grade 2) in the entire cohort were identified. Confirmation of the 43 suspected asymptomatic dengue infections and the determination of the infecting serotypes using the plaque reduction neutralization test are ongoing. During 2007 and 2008 the estimated incidence of asymptomatic dengue infection in this cohort was 44.4/1,000 populations per year compared to an incidence of symptomatic infection of 10.7/1,000 populations per year.

3043

Molecular Basis of Divergent Unstable Actins in Apicomplexan Parasites

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Apicomplexan invasion of host cells involves a unique gliding motility mechanism that is dependent on polymerization of parasite actin. *T. gondii* actin, TgACTI, is highly divergent from conventional actin and only polymerizes into short, unstable filaments that exhibit rapid turnover kinetics. *P. falciparum* has two actins, PfACTI and PfACTII, which also diverge from conventional actin and behave similar to TgACTI in their polymerization properties. The instability of parasite actins appears crucial for proper motility as inhibiting actin filament turnover disrupts normal motility and cell invasion. To determine what contributes to this unusual polymerization, we examined parasite actin filaments using molecular modeling, molecular dynamics and biochemical assays to monitor polymerization. Molecular modeling demonstrated that parasite actins contain a conserved phalloidin-binding site bridging three subunits within the filament. Consistent with this, phalloidin rescues the short filaments formed by parasite actins, as visualized by fluorescence and electron microscopy. These results demonstrate that although parasite actins naturally form unstable filaments, they are capable of forming longer, conventional filaments when stabilized. Molecular dynamics and docking identified residues unique to apicomplexan actin that may impact filament stability. Critical substitutions were revealed in the hydrophobic plug and phalloidin-binding pocket in parasite actins that are predicted to affect subunit interactions within the filament. To test this model, residues unique to apicomplexans were replaced within TgACTI with those from muscle actin. Fluorescence microscopy and light scattering polymerization assays demonstrated that these changes increased stability of TgACTI. These findings indicate that the rapid turnover of parasite actins is due to a small number of molecular differences that affect stability. Stabilized actin is predicted to disrupt gliding motility and this is currently being tested by overexpression of mutant TgACTI alleles in the parasite.

3044

Immune Response to Cryptosporidium parvum in University Students and HIV-positive Subjects in the Venda Region, Limpopo Province, South Africa

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Cryptosporidium is one of the most prevalent etiological agents of persistent diarrhea worldwide. Previous studies conducted in the Venda region have shown a stool PCR prevalence of 16% among children and 12.5% among HIV-positive individuals. The actual exposure rate to this pathogen, however, would be expected to be higher. Immunological studies of immunocompetent individuals suggest that the immunopathogenesis to *Cryptopsoridium* infection is predominantly via cell-mediated immunity (CMI). High levels of IFN-γ are produced in humans recovering from infection. Some preliminary studies utilizing IFN-γ release assay (IGRA) technology have demonstrated IFN- γ production after stimulation with *C. hominis* antigens in healthy seropositive volunteers. In the present study, the seroprevalence of *Cryptosporidium parvum* infection in the Venda Region in South Africa is determined. In addition, we determined the IFN- γ production of 12 healthy students using the QuantiFERON CMI-Kit assay (Celestis) stimulated with excysted *C. parvum* antigens.

Using a cohort of 234 HIV-positive subjects from the Venda region and 57 healthy students at the University of Venda in Limpopo, a screening ELISA using purified excysted *Cryptosporidium parvum* antigen (CCE) (Iowa isolate; Waterborne, Inc., New Orleans, Louisiana) was performed to identify the presence of IgG. Using a cutoff point of 1.8*OD of the negative control, 41% of students and 71% of the HIV-positive cohort were positive ($p<0.01$, Chi-Square). As determined by analysis with the QuantiFERON software, two of five seropositive and three of six seronegative students were positive for IFN- γ production upon exposure to the excysted antigen. This study demonstrates a higher prevalence of *Cryptosporidium* infection in the Venda region, especially among the HIV-positive population, than was previously known. The discordant response between ELISA and IGRA to *C. parvum* CCE assays demonstrates the complexity of the immune response to *C. parvum* CCE warrants further investigation.

3045

Functional analysis of dengue virus non-structural 4B protein using reverse genetics

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The four serotypes of dengue virus (DENV, genus *Flavivirus*) carry a single-stranded, positive-sense RNA genome that codes for 3 structural and 7 nonstructural (NS) proteins. The 245 amino acid (aa) NS4B protein is part of the DENV replication complex, however genetic analysis of the role of NS4B in viral replication is lacking. To investigate NS4B function, a series of mutations were introduced into full-length DENV-4 cDNA using reverse genetics. First, to define the minimal NS4B sequence required for viability, N-terminal deletions of 30, 75, 120 and 240 aa were generated, but viable virus was not recovered following transfection of any of the resulting RNA constructs in either mosquito (C6/36) or monkey kidney (Vero) cells. DENV-4 proteins were produced by all constructs, but DENV-4 RNA was not detected from any construct, suggesting that NS4B is critical for replication but not translation of the viral genome. Second, NS4B aa residues 101, 109, 112, 119 and 240, previously shown to be the sites of Vero cell-adapting mutations, were individually deleted. Viable virus was not recovered from any of the resulting constructs except rDENV-4Δ101, which was recovered in C6/36 but not Vero cells. Third, the same aa set were mutagenized individually to Ala. In C6/36 cells, all mutant viruses except rDENV-4Pro101Ala were recovered; in Vero cells only rDENV-4Pro101Ala and rDENV-4Gly119Ala were recovered, albeit at low titers. The Val109Ala and Gly119Ala mutations did not affect replication kinetics in C6/36 cells, but rDENV-4Leu112Ala showed significantly enhanced replication kinetics relative to wild type DENV. Finally, to test whether the loss of NS4B in rDENV-4 could be complemented *in trans* by NS4B from another DENV serotype, Vero cells were infected with DENV-2 and transfected 2 hours post-infection with the four DENV-4 deletion constructs described above; control cells were mock-infected and then transfected with each construct as above. DENV-4 RNA was recovered from all treatments infected with DENV-2, but not from control transfections, 72 hours post-infection.

3046

Colonization, Life Tables and Evaluation of Two Artificial Diets of the Blowfly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), Bogotá-Colombia Strain

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The objective of this work was to establish, under experimental laboratory conditions, a colony of *Lucilia sericata*, Bogotá-Colombia strain, to build life tables and to evaluate two artificial diets. This blowfly is frequently used in both post-mortem interval studies and in larval therapy. The parental adult insects collected in Bogotá were maintained in cages at 22°C±1 average temperature, 60%±5 relative humidity and 12 h photoperiodicity. These blowflies were fed on two artificial diets which were evaluated through seven continuous generations. Reproductive and population parameters were assessed. The life cycle of the species expressed in days through the different stages was: egg= 0.8±0.1, larvae I= 1.1±0.02, larvae II= 1.94±0.16, larvae III= 3.5±0.54, pupae= 6.55±0.47, male adult= 28.7±0.83 and female adult= 33.5±1.0. Total survival from egg stage to adult stage was 91.2% for diet 1, while for diet 2 this parameter was 40.5%. The lifetime reproductive output was of 184.51 eggs per female. The population parameters as well as the reproductive output from the blowflies, that were assessed, showed relatively high values giving evidence of the continuous increase of the strain through the different generations and making possible its maintenance as a stable colony which has lasted for more than two years.

3047

Liver proteome analysis during progressive pathology in murine schistosomiasis

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Schistosoma mansoni associated hepatomegaly is estimated in 8.5 million people. Liver granulomas formed due to trapped parasitic eggs are involved in the development of pathology. After infection, individuals suffer from acute toxemic schistosomiasis at 6-8 weeks post infection which progresses to a 4-5 year chronic schistosomiasis. While curative therapies are available, re-infection is common. Chronic schistosomiasis presents with either a moderate or a severe form, termed intestinal or hepatosplenic schistosomiasis, respectively. The CBA/J mouse model replicates these disease forms and thus can be used to understand the progressive pathology that leads to hepatosplenic schistosomiasis. Using this model, we compared the liver protein patterns of control mice and mice infected for 6, 8, 12, or 20 weeks. Two dimensional differential in gel electrophoresis was used to identify protein pattern variations and revealed 76 significant protein spot changes of which 44 protein spots were identified using MALDI-TOF mass spectrometry. Of these changes, we found that the abundance of keratin D, transferrin isoforms and *Schistosoma mansoni* phosphoenolpyruvate carboxykinase increased while peroxiredoxin 6, major urinary protein isoforms and carbonic anhydrase III isoforms decreased

significantly. Furthermore, mouse serum major urinary protein was decreased ($p<0.01$) and serum transferrin was increased ($p<0.01$) in mice with severe hepatosplenic disease when compared to uninfected mice or infected mice with moderate disease. We anticipate that these findings can be used to develop diagnostic tools for early detection of hepatosplenic schistosomiasis in humans.

3048

Detection of Old World Hantaviruses in New Orleans, Louisiana

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Hantaviruses are segmented, negative-sense RNA viruses of the family *Bunyaviridae* that are maintained in nature by rodents, generally with a tight virus/rodent reservoir pairing. The geographic distribution of a given hantavirus is thus generally dictated by the distribution of its specific rodent host. Two distinct diseases are associated with human hantavirus infection: hantavirus pulmonary syndrome from New World hantaviruses and hemorrhagic fever with renal syndrome (HFRS) from Old World hantaviruses. Despite their name and evolutionary origin, some Old World hantaviruses, such as Seoul virus, can be found worldwide, most likely through spread of their rodent reservoirs on ships. Thus, rodents and humans in port cities are of particular interest with regard to Old World hantaviruses and HFRS. We conducted a survey of Old World hantaviruses in rodents in the port city of New Orleans, Louisiana. Rodents were trapped, mainly in the city center, using Tomahawk traps and their locations recorded by GPS over 12 months. One hundred and twenty-one rodents were trapped over 45 trap nights (8.9% trap success), of which 100 (83%) were *Rattus norvegicus*. Captured rodents were euthanized and necropsies were performed using standard procedures. Reverse-transcriptase polymerase chain reaction was performed on RNA extracted from homogenized lungs using primers known to amplify a 280 base pair conserved region of the nucleocapsid gene of the S segment. To date, one *R. norvegicus,* an adult female, has tested positive. Preliminary sequence analysis revealed 99% nucleotide homology with a Seoul virus variant found in New Orleans and designated Tchoupitoulas virus by Tsai *et al*. in 1985. These data suggest that Tchoupitoulas virus has persisted in New Orleans over the last 25 years, although it remains to be determined whether it is associated with human disease. We plan to continue surveillance for Old World hantaviruses as well as novel pathogens in New Orleans and also conduct serological surveys to assess exposure of Tchoupitoulas virus in humans.

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Role of ClipA8 in *Plasmodium yoelii* melanization in *Anopheles gambiae*

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Understanding mosquito malaria interactions would facilitate developing new measures intervening malaria transmission. *Anopheles gambiae* is partially refractory to murine malaria *Plasmodium yoelii*. In this study we demonstrated that around 50% mosquitoes were able to melanize the ookinetes and/or early oocysts of *P. yoelii*. The melanization requires serine protease gene *ClipA8*. Silencing *ClipA8* via RNAi abolished the parasite melanization. Intriguingly, the abolishment of melanization did not affect the live oocyst load, the prevalence and intensity of live parasites were similar between the *ClipA8* knockdown and control mosquitoes, suggesting that melanization mechanism is not involved in parasite killing; instead it participates in the process of cleaning dead parasites that were killed by different mechanism(s). This phenotype resembles the *Plasmodium* melanization in refractory L3-5 strain of *An. gambiae.*

3050

Novel boron-containing small molecules demonstrate potent *in vitro* activity against *Plasmodium falciparum* with excellent drug-like properties.

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There is an urgent need to discover new medications to treat falciparum malaria. New drugs must counter resistance to older drugs, be active orally, be effective in short-course therapy, be relatively inexpensive to produce, and be safe for use in developing world populations. We have developed boron-containing compounds with potential as anti-parasitic drugs, with potent activity, specificity and excellent drug-like properties. A panel of 1,100 oxaborole compounds was screened *in vitro* against *P. falciparum*. This resulted in 42 potent hits, with IC_{50}s below 1 μM. Three unique chemical scaffolds were prioritized. IC_{50}s for the best compound in each of these series were 26 nM, 52 nM and 156 nM, respectively. For the best compound, AN3661, with an IC_{50} of 26 nM, further experiments were conducted to assess drug-like properties, including solubility, cytotoxicity and pharmacokinetic profile in mice. AN3661 is soluble at 750 μg/ml in 10 mM PBS at pH of 7.0. A safety index of >100 fold was observed in human KB carcinoma cells,

murine J774 macrophages, and murine L929 fibroblasts. Pharmacokinetic studies of AN3661 showed an oral bioavailability of 53%, an $AUC_{0\text{-}inf}$ of 3.71 h*µg/ml, a C_{max} of 2.66 µg/ml, and a $t_{1/2}$ of 1.42 hours; all leading to an oral exposure, when dosed at 30 mg/kg, that was well above the IC_{50} for greater than 8 hours. Blood-to-plasma partitioning of AN3661 in non-infected mice showed equal distribution between blood and plasma, a 93% blood:plasma ratio. Lead compounds are progressing to *in vivo* efficacy studies. Our preliminary results suggest that new oxaboroles have excellent potential as novel antimalarial agents.

3051

Vaxfectin® enhances antibody and T-cell responses of malaria plasmid DNA vaccines administered at low doses in nonhuman primates.

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Despite demonstrated efficacy in rodent models, nonadjuvanted plasmid DNA (pDNA) vaccines have modestly efficacious in nonhuman primates and in human clinical trials. In the *P. yoelli* rodent model for malaria vaccine development, we have previously reported that Vaxfectin®, a cationic lipid delivery system, significantly enhances the potency of a malaria pDNA vaccine administered at low doses which reflect dosages used in human clinical trials. In the present study, we demonstrate enhancement of both antibody and T-cell responses in *Macaca fascicularis* monkeys immunized with low (150 ug) or high (750 ug) doses of *P. falciparum* CSP and AMA-1 pDNA formulated with or without the adjuvant Vaxfectin®. Faster induction of antibody responses to both antigens was observed in monkeys immunized with pDNA formulated with Vaxfectin® as compared to PBS (0/16 vs 5/16 for CSP, 0/16 vs 15/16 for AMA). The number of responders remained consistently higher in Vaxfectin® immunized groups following subsequent immunizations with pDNA and recombinant adenovirus, and antibody titers to both antigens were consistently higher with Vaxfectin® formulation and were statistically significant for AMA-1 responses. Although differences in IFN-gamma T-cell responses were not observed between groups following immunization with pDNA alone, the proportion of monkeys with positive IFN-gamma responses to both antigens was higher in Vaxfectin®-immunized groups (2/16 vs 5/16 for CSP, 2/16 vs 15/16 for AMA) following boosting with recombinant adenoviruses, although group differences in the magnitude of IFN-gamma SFCs did not reach statistical significance. These studies suggest that Vaxfectin® may be a useful adjuvant for enhancing pDNA malaria vaccine in humans.

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New Vi-CRM_{197} vaccine against typhoid fever with good potential for industrial scale development

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Salmonella enterica serovar Typhi causes typhoid fever with over 22 million cases reported annually, resulting in ~200,000 deaths. *S.* Typhi expresses the capsular polysaccharide Vi, which is the target of immune protection. Licensed Vi-based vaccines provide ~70% protection in adults but, being a T-cell independent antigen require a booster dose every 3-5 years and do not induce good immune responses in children below 2 years of age. Several Vi-protein conjugate vaccines (e.g., Vi coupled to recombinant *Pseudomonas aeruginosa* exoprotein A, Vi-*r*EPA, or tetanus toxoid) have been developed and clinical trails have shown the Vi conjugate to have protective efficacy in older children, and to be immunogenic in infants. The Novartis Vaccines Institute for Global Health is developing a new Vi-conjugate vaccine based on Vi derived from a genetically unmodified *Citrobacter* and CRM197, the mutant diphtheria toxin protein. Vi from *Citrobacter*, a low risk organism, is structurally similar to *S.* Typhi Vi. CRM197 is the carrier protein in several licensed polysaccharide vaccines. A *Citrobacter* capable of stable production of high Vi levels was selected for development and cGMP manufacture. Cell banks were prepared and fermentation in chemically defined media was optimized to obtain reproducibly high yields of Vi in a 50 L bioreactor. An efficient, gentle, economical and scalable process for Vi purification was developed that retains high levels of O-acetylation. Conjugates were prepared by carbodiimide-mediated synthesis using adipic acid dihydrazide derivatized CRM197 and Vi activated with EDAC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride). Well characterized conjugates were used to immunize mice and rabbits for the evaluation of antibody responses. Vi-CRM197 conjugates were well tolerated and elicited high anti-Vi and anti-CRM197 antibody levels in animals. This data supported the ongoing cGMP manufacture of the Vi-CRM197 conjugate. A GLP toxicology study in rabbits is scheduled for later this year and a Phase 1 trial is planned for 2010.

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Knowledge, Attitudes, Practices and Perceptions (KAP) on ITNs, IRS and other known traditional preventive methods for Malaria Control in Zambia

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Background:
Malaria has for a long time been a major public health concern in Africa and Zambia in particular. Due to the endemic and persistent nature of Malaria in Zambia, the government through The National Malaria Control Center (NMCC) has in the recent past introduced on-going programs aimed at reducing the incidence of malaria for the sore purpose of meeting the demands for all socio-economic classes and communities. Insecticide-Treated Nets (ITNs) and In-door Residual Spraying (IRS) are the primary control strategies for preventing malaria transmission in Zambia though there are other known traditional preventive methods that the community engage in. The results of the 2006 first nationally represented household survey of key malaria interventions and malaria-related burden among children aged under five revealed that 50.1 per cent of Zambian households had at least one mosquito net.
Methods:
This study was cross-sectional in nature and was done at household level in all the nine provinces of Zambia in order to assess the Knowledge, Attitude, Perceptions and Practices (KAPs) levels for malaria control services in Zambia. This paper focuses on known malaria preventive methods and findings in Zambia.
Results:
The knowledge levels on causes of malaria were very high with 79% of the respondents indicating that malaria is caused by mosquito bites. 66% indicated that sleeping under a net is the best known method on which one can protect themselves against malaria of which 41% was an ordinary net and 25% was an insecticide. The research revealed that, where there is only one net in a household, the net was mostly used by an under five child and a pregnant woman with 59% and 34% respectively. The study further revealed ownership of nets of more than 80%. Twenty Four (24%) of the respondents indicated that they own at-least one net, where as 27% indicated that they had atleast two nets. Sixteen percent (16%) and 17% of the respondents indicated that they had atleast three nets and four nets respectively. Furthermore, it was good to not that most of the mosquito nets in the communities where used for protection against mosquito bites (87%).
Conclusion:
The knowledge levels in Zambia indicated that the use of mosquito nets is the most form of known preventive methods against contracting malaria. This was not surprising as the Ministry of Health through the National Malaria Control Centre (NMCC) and its partners embarked on mass distribution of ITNs.

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Prevalence of Uncommon Enteric Pathogens Among Egyptian Children with Severe Diarrhea

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A hospital-based study was conducted in Egypt to determine etiology and incidence of enteric pathogens in children < 5-years of age. Initial assays (standard bacteriological methods, commercially available enzyme immunoassays (EIA) for *Cryptosporidium* and rotavirus) detected a pathogen in nearly 60% of cases. We subsequently added five EIAs for enteric pathogens (adenovirus, astrovirus, norovirus, *Giardia lamblia* and *Entamoeba histolytica*) to determine their prevalence among enrolled cases.
From 2005 to 2007, stools from 2112 children with diarrhea from Mokatam Hills (urban) and Abu Homos (rural) were collected. Frozen stool samples were tested for adenovirus, astrovirus, norovirus, *G. lamblia* and *E. histolytica* using commercially-available EIA kits.
With respect to sole pathogen-associated diarrhea, 2% (n=34) of the cases were infected with adenovirus, 3% (n=56) with astrovirus, 9% (n=191) with norovirus, 7% (n=146%) with *G. lamblia*; 14% (n=299) were mixed infections. Collectively statistical analyses of all pathogens showed the percentage of diarrheal cases with known causes increased from 48% (n= 1006) to 74% (n= 1568) including 40 % (n= 853) sole pathogen and 34% (n= 715) mixed infections. Diarrhea cases with bacterial infection as the sole pathogen decreased from 20% (n= 428) to 10% (n= 202) while virus-associated and parasitic-associated diarrhea increased from 4% (n= 286) to 21% (n= 452) and 5% (n= 96) to 9% (n= 199) ($p < 0.0001$), resepctively. The highest of enteric pathogens was detected in children aged < one year. Comparing cases with single infection in the two studied sites, we found that parasitic (60%; n=120/199), viral (61%; n=277/452) or mixed (64%; n=459/715) infections were higher in rural areas as compared to urban area.
Data obtained in this study further demonstrates the highest % of diarrheal infection in pediatric cases in Egypt occurs during the first two years of life. Moreover, identification of polymicrobial infection (viral plus bacterial or parasitic) in cases with pediatric diarrhea is not uncommon and warrants further study.

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The first 100 cases of Pandemic Influenza A (H1N1) in Kenya

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Abstract
Introduction

The 2009 pandemic influenza A/H1N1 (pH1N1) has infected over one million people worldwide and caused 3,486 deaths. As of September 14, 2009, 24 (52%) of countries in Africa had reported cases of pH1N1 with very limited reported information on clinical and epidemiologic characteristics of cases. We describe the clinical and epidemiologic characteristics of the first 102 cases of laboratory confirmed pH1N1 in Kenya.
Methods
In May 2009, the Kenya Ministry of Public Health and Sanitation expanded existing surveillance for influenza to 29 hospitals and clinics throughout Kenya. A suspected pH1N1 case was defined as someone with acute febrile illness and an epidemiologic link to a known pH1N1 case. Nasopharyngeal and oropharyngeal samples were collected from suspected pH1N1 patients at sentinel sites and from suspected patients who had links to a known outbreak. Epidemiologic and clinical data were collected using standardized data forms. Specimens were tested at the National Influenza Center and the Center for Disease Control and Prevention - Kenya by RT-PCR for pH1N1 and other influenza viruses.
Results
The first pH1N1 case in Kenya was confirmed on June 29, 2009. From April 29 through September 1, 2009, 667 suspected cases were tested, of which 102(15.3%) were positive for pH1N, 21(3.1%) were positive for seasonal H1N1, 16(2.4%) for H3N2 and 15(2.2%) for Influenza B. The mean age of positive cases was 26 years; 33(32.4%) cases were 11-20 years old, and 30 (29.4%) were 21-30 years old. Among 87 cases with available clinical information, 20(22%) had an underlying medical condition; of these, 13(15%) had asthma. Among 84 cases with available history on travel and contact with known cases, 53(63%) had no history of travel and 51(54.8%) had contact with confirmed pH1N1 cases. Contact with confirmed pH1N1 cases was reported in 51(54.8%) cases. Ten patients (10%) were hospitalized. No deaths occurred.
Conclusion
Community transmission of pH1N1 is established in Kenya. Demographics and clinical characteristics of pandemic H1N1 patients in Kenya are similar to those reported in other countries. In Kenya, from May-September 2009, pH1N1 was not as overwhelmingly predominant as it was in temperate climates, and seasonal influenza viruses co-circulated.

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Distribution of *Aedes albopictus* larval habitat at various altitudes in Republic of Korea

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Distribution of *Aedes albopictus* larval habitat at various altitudes in Republic of Korea
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Abstract
In Korea , Ninety eight patients infected with dengue fever were reported in 2007.
Ninety percent of the cases have been imported from other South-east Asia and Pacific islands during summer season. Several disease vectoring arthropods are known to occur during summer in Korea. One of the known disease vector has been *Aedes albopictus* in rural to urban areas. This species is a occasional vector of dengue viruses in parts of Asia , besides being a competent vector for several other viruses. The Korean peninsula has been experiencing longer summer seasons over the past few years , that represents an indications of possible climate change.
It is possible that this may cause serious outbreaks of vector borne diseases in Korea.
In order to know the distributional pattern of *Aedes albopictus* with particular reference to climate change, we conducted entomologic studies from June to August, 2009, that focused on: To identify disease vectors and other mosquitoes in the surrounding rural and mountain areas. To survey mosquito breeding sites around human habitation and temple area.
Out of the total area surveyed 210 were found to be potential mosquito breeding areas . They were surveyed between altitudes of 26 and 1,244m.
We identified over 3,000 mosquito specimens and classified there in 6 genera 14species.
Therefore this study marks the importance of the change in climate that may erupt in more mosquito breeding in our region. It is proposed to have continuous monitoring of mosquitoes at various altitudes of Korea and observe their dispersal to the human active zones.

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Preventing Malaria in Rural Africa with Durable Linings (DL) - a sustainable and effective alternative to Indoor Residual Spraying (IRS)

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INTRODUCTION
Community level malaria protection requires coverage and usage of an effective tool by >80% households. Dependency on Indoor residual spraying (IRS) is increasing because impact is not dependant upon behaviour change. However, IRS requires household acceptance for access, extensive campaign planning and technical skills together with sustained political organisational and logistical commitment and capacity. Sustaining household acceptance for repeat IRS is challenging as perceived benefit to households may

reduce over time. Durable Lining (DL) may eliminate future need for IRS in rural communities.

METHODS

DL is a dual purpose tool for home improvement and malaria prevention. DL material combines aesthetic decorative values with vector control performance based on IRS principles and adapting technology developed originally for long lasting nets. Field trials established in Nigeria in 2006 and Equatorial Guinea, Kenya, Angola, Mali, Ghana, South Africa, and Vietnam in 2008 are evaluating installation, durability, acceptability and malaria related outcomes of DL. Baseline surveys were conducted prior to installation, followed by periodic surveys of acceptance, material and residual chemical durability, and malaria indicators over a minimum period of 9 months.

RESULTS

Study results show higher user acceptance for DL than for IRS, and good material durability in all studies. Bioefficacy (by WHOPES test protocol) exceeded minimum requirements in all trials. High bioefficacy was sustained on house construction materials including wood, mud, brick and concrete over the full monitoring period in each trial to date and far exceeded the residual impact of IRS. In Nigeria, DL remains effective >2 years after installation and malaria prevalence has reduced significantly in Angola 10 months post installation.

DISCUSSION

Studies confirm that DL is a more suitable, acceptable, effective and sustainable malaria prevention tool than IRS for use amongst stable rural communities living in malaria endemic areas.

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Antiplasmodial activity of a small imidazolium-based compound

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The emergence and spread of resistance to traditional antimalarial agents has created an urgent need to develop antimalarial agents that use novel mechanisms to treat the disease. We are currently studying a compound which contains a phenyl and napthyl group linked to a central imidazolium ring (designated QT69) in order to examine the antiplasmodial activity it has shown in culture. Specifically, this compound is effective against *Plasmodium falciparum* at an IC_{50} of 0.9 ± 0.2 uM, while having an IC_{50} of 108 ± 6 uM in Chinese hamster ovarian cells, thereby suggesting a direct antiparasitic effect. We have also tested the effectiveness of QT69 in an *in vivo* mouse model. Balb/C female mice were infected with *P. berghei* and treated with either an isotonic control or 18ug QT69 once a day for three days. Compared to control mice, mice treated with QT69 showed a significant suppression of parasitemia during the time of the infusions ($P<0.1$). To determine if QT69 was targeting a parasite component as opposed to a host red blood cell (RBC) component mature-stage parasitized RBCs (pRBCs) or uninfected RBCs (nRBCs) were treated with QT69 for a period of 3 hours. The compound was then washed out and parasite viability was determined 48 hours later. Treatment of pRBCs, but not nRBCs resulted in a reduction in parasite viability. We therefore conclude that QT69 affects a parasite component. We were interested in identifying at what stage in the parasite's life cycle QT69 is having its effect. Our preliminary TUNEL assay results suggest that QT69 does not kill schizont-stage parasites; instead it appears to be killing parasites at a time point between their schizont stage and their young trophozoite stage. Flow cytometry data has shown that upon treatment with QT69 there appear to be 3 cell populations: uninfected RBCs, parasitized RBCs, and a population with properties consistent with free merozoites, suggesting that treatment with QT69 may be inhibiting the ability of merozoites to reinvade RBCs.

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TSS-seq: A powerful new method for transcriptome analysis

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TSS-seq is a novel method to assess the frequency of expressed genes as well as to analyze the exact transcription start sites at an unprecedented level by combining the oligo-capping method and the ultra-high speed Solexa sequencer. Millions of short sequences of the very 5'-end of mRNAs are determined in a cost-efficient way and mapped to the respective genome sequences, enabling detailed expression profile analysis. We have applied this method to malaria and toxoplasma parasites as well as to vector arthropods, including anopheles mosquitoes and tsetse flies. The extreme power enables to analyze not only stage transition, i.e., bradyzoite induction in toxoplasma parasites, cyst induction in Entamoeba and Giardia parasites but also host-parasite interaction, leading to elucidation of pathogenesis and immunological responses in patients. Another interesting use is the analysis of extremely short 5'UTR (untranslated region) of Entamoeba and Giardia parasites. Preliminary results are available at Full-Parasites and Full-Arthropods (http//:fullmal.hgc.jp). This method should revolutionize research of parasitology and entomology.

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High prevalence of asymptomatic malaria in southeastern Bangladesh

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Due to spreading resistance of Plasmodium falciparum to existing drugs the malaria situation in Bangladesh may worsen, particularly in the Chittagong Hill Tracts, where surveillance and research data about malaria remain scarce.
A cross-sectional survey was conducted in Bandarban, one of three Hill Tracts Districts, the region with the highest malaria endemicity in Bangladesh. Blood smears were obtained from all participants and diagnosed using microscopy and rapid diagnostic tests. Hemoglobin, temperature, spleen rate and demographic data were recorded. Malaria prevalence, parasite density, prevalence of anemia, bed net use and distance to forest were assessed to provide baseline data for improving malaria control programs in the country.
The mean malaria prevalence in the summer survey (rainy season) was 14.4% (95% CI: 12.7 - 16.4) with an upper limit at village level of 33.3%. P. falciparum represented 78.7% of all infections, P. vivax 19.3%, and 1.9 % were mixed infections. The proportion of asymptomatic infections was 71.0%, the oligosymptomatic and symptomatic cases represented 25.1% and 3.9%, respectively. Malaria prevalence and parasite densities were significantly ($P<0.001$) higher in patients younger than 15 years. Spleen rate and malaria prevalence in 2-9 years olds were 18.4% and 25.7%, respectively.
The overall prevalence of anemia was 72.4% and an association with malaria was found in children 8-12 years old and females older than 15 years. Bed net availability was 51.0%, however only 25.2% were treated with insecticides in the last 6 months.
With only 7.7% (34/439) the malaria prevalence in a winter survey (dry season) was significantly ($p<0.001$) lower.
This cross sectional survey showed a large reservoir of asymptomatic plasmodium infections during the monsoon months, which likely acts as a source of transmission. New strategies for malaria control may be needed in this region, where malaria control is currently based on the treatment of symptomatic patients. This situation highlights the need to develop new intervention strategies specifically targeting asymptomatic carriers.

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Sensitivity and repeatability of Kato-Katz, McMaster and sedimentation methods for quantification of hookworm and Ascaris fecal egg counts

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Clinical manifestations of intestinal helminthiases are related to infection intensity which, in clinical practice and research, is commonly estimated via fecal egg counts. Two stool samples were taken, 10 days apart, from each of 176 people from a rural area of Minas Gerais State, Brazil. They were examined for hookworm and Ascaris eggs by Kato-Katz (KK), McMaster (MM) and sedimentation methods, by a team of four readers. Each test was performed on two subsamples of each sample. Analysis was done on the total egg count over two or four replicates per subsample for KK or MM, respectively. Both totals correspond to approximately 1/12g of stool (2/24 or 4/50g, respectively). KK had greater between-reader variation than MM: markedly so for hookworm (repeatability of square root counts 10.5 versus 3.9) and to a lesser extent for Ascaris (25.6 versus 19.2). For both species, KK yielded higher egg counts than MM, despite being based on equal stool mass. For hookworm, the mean difference (KK minus MM) between the methods was 1.54 (95% CI 1.47-1.61) times their consensus value. Accordingly, more subsamples tested positive on KK than on MM. For hookworm, the number of positive subsamples (0, 1 or 2) differed between KK and MM in 16 of 162 samples: in 13 of these (81%, 95% CI 54-96%) it was KK rather than MM which had the greater number of positives. The difference was even more pronounced for Ascaris: 42 of 98 samples differed in numbers of positive subsamples, and in 40 of these (95%, 95% CI 84-99%) KK had more. For no combination of species and method was there evidence of systematic differences between readers. Finally, a maximum likelihood method is presented for estimating egg density when sedimentation is positive but KK or MM is negative. These results suggest that, even after taking into account its greater stool volume per replicate, KK is more sensitive than MM, an advantage which may outweigh greater between-reader variation.

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Allelic heterogeneity underlying glucose-6-phosphate dehydrogenase deficiency in malaria-endemic regions

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme deficiency in man, affecting approximately 400 million people worldwide. It is among the most heterogeneous genetic traits known, with hundreds of distinct functional variants of varying penetrance characterized worldwide, many of which have achieved polymorphic frequencies. As geographic distribution of G6PD deficiency strongly colocalizes with malaria endemicity, it has long been thought to be a protective trait under strong positive selection.
In spite of nearly a half-century of research, however, several fundamental questions persist regarding the relationship between G6PD deficiency and malaria. Such questions include: the range of functional genetic diversity at the G6PD locus, the extent and selectivity of a malaria-protective effect, and the molecular mechanism(s) underlying clinical protection.
MalariaGEN, an international consortium comprised of researchers from twenty-one countries, is dedicated to using the tools of genomic epidemiology to discover and understand host protective mechanisms that may influence morbidity and mortality in severe malaria. We present here preliminary data from resequencing and dense genotyping at the G6PD locus in a MalariaGEN cohort comprised of individuals from twelve malaria-endemic countries. We find considerable genetic heterogeneity in both coding and noncoding sequence, and allelic heterogeneity underlying biochemical deficiency of the enzyme, observations that may have important implications for design of population genetic and disease association studies at this locus.

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Negative impact of polyparasitic infection on growth, anemia, and physical fitness among children in coastal Kenya

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To better quantify the disease burden of concurrent parasitic infections in a poly-endemic area of Kenya, we examined the individual and combined associations between schistosomiasis, filariasis, malaria, and geohelminth infection with abnormal growth-related anthropometrics, low hemoglobin levels, and low fitness scores among resident children, ages 5-19. Beginning February 2009, cross-sectional data were obtained from a community-wide survey of Nganja, Kenya including demography, household SES and health knowledge. Results for 240 children revealed a high prevalence of schistosomiasis (62%), trichuris (37%) and hookworm (22%). Malaria prevalence was relatively low (8%) as was filariasis (6%). Co-infection was most frequent for schistosomiasis-trichuris (24%) followed by schistosomiasis-hookworm (15%). By Hemocue photometer, and using the recently developed WHO growth standards for 5-19 yo,anemia (47%), stunting (57%) and wasting (35%) were all highly prevalent. Nine percent were severely malnourished (-3SD in BMI-for age).There were strong associations between M+ category (high/medium intensity) *S. haematobium* infection and both anemia and severe malnutrition. These associations were not affected by concurrent infection with*Trichuris,* hookworm or malaria. Filaria positive children were more likely to be wasted (-2 SD in BMI-for-age, OR=2.9), with greater odds in the presence of M+ schistosomiasis (OR=3.5). When controlling for age, anemia was a predictor of wasting, and both these factors, as well as hookworm infection, were associated with lower fitness scores. We hypothesize that even in the absence of clinical illness, infection and co-infection with these parasites impairs the normal growth of children and contributes to anemia of inflammation resulting in chronic disability and decreased adult productivity. New data collection from other villages is in progress and links between chronic and acute undernutrition and micronutrient deficiencies as well as pro-inflammatory cytokines are also being explored.

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Memory CD4+ T cell responses against Plasmodium falciparum Merozoite Surface Protein-1 in vaccination and naturally acquired immunity

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The *Plasmodium falciparum* Merozoite Surface Protein-1 (MSP1) is a leading malaria vaccine candidate because it has been shown to confer protection in pre-clinical models. Immune responses to MSP1 have also been consistently observed in individuals living in malaria endemic areas. Previously we showed that CD4+ T cell responses elicited by vaccination with the C-terminal $MSP1_{42}$ were primarily localized to the $MSP1_{33}$ region, and we have now characterized the induction of memory CD4+ T cells after vaccination with $MSP1_{42}$-C1 (a combination of the FVO and 3D7 allelic forms of the protein). Further, we have classified $CD4^+$ memory T cell responses as effector memory T cells (T_{EM}), central memory T cells (T_{CM}), and effector memory T cells bearing isoform CD45RA (T_{EMRA}) based on phenotypic characteristics. We have characterized the contribution of these different memory T cell populations following $MSP1_{42}$ vaccination of malaria naïve volunteers with two different antigen doses (40μg and 160μg) in two different formulations, Alhydrogel and Alhydrogel plus the TLR9 agonist CpG. The higher antigen dose formulated with Alhydrogel plus CpG elicited greater ex vivo production of IL-2, IFN and TNF in supernatants of peripheral blood mononuclear cells obtained from vaccine recipients and restimulated in vitro with homologous antigen. T_{EM} and T_{CM} (but not T_{EMRA}) populations were identified and characterized following vaccination. The memory responses to $MSP1_{42}$ vaccination were compared with those elicited by natural infection in Malian adults; both T_{EM} and T_{CM}cell responses were found to be significantly greater in Malian adults as compared to vaccinees. These findings confirm that $MSP1_{42}$ vaccination induces a variety of $CD4^+$ memory T cell responses, but further studies

will be needed to determine whether these responses are involved in reducing parasite densities and the incidence of malaria in endemic areas. Information obtained from this study could help in the rational design of malaria vaccines.

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Computational network analysis predicts antileishmanial activity of FDA approved drugs

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A genome-scale metabolic network reconstruction of *Leishmania major*, the first of its kind for a protozoan, was recently published. The metabolic network accounted for 560 genes and consisted of 1112 reactions across 8 sub-cellular compartments. Using flux balance analysis (FBA), the computational model enabled the prediction of essential genes that could serve as potential therapeutic targets. In order to further prioritize our drug target predictions, we used a druggability index from tdrtargets.org and sequence similarity to known protein targets linked to Food and Drug Administration (FDA) approved drugs obtained from drugbank.ca. Of the 560 genes accounted for in the *L. major* metabolic reconstruction, we identified 17 high priority targets associated with 79 approved drugs. Predicted in the list of 17 genes are some established trypanosomatid targets such as trypanothione reductase and ornithine decarboxylase. Drugs identified in this analysis with high acute toxicity tolerance levels (LD50) are currently being evaluated *in vitro* for novel antileishmanial activity. As an example, the IC50 of disulfiram (marketed as Antabuse or Antabus), a drug with an LD50 of 8600 mg/kg (rat; oral) and used in the treatment of alcohol abuse, was in the low micromolar concentration range when evaluated against *L. major* using the alamarBlue assay. Separately, through network analysis, we have also identified conditionally essential target genes, whose essentiality could be manipulated by constraining an environmental input, thus providing for an additional therapeutic strategy. Hence, metabolic network analysis and prioritization of targets using bioinformatics has led to the identification of several novel drug targets associated with a list of approved drugs that may be efficacious against a critically important infectious disease.

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Investigation of Rickettsial Vectors and Reservoir Hosts in Military Areas of Operation (AOs) along Northern Thai-Myanmar and Thai-Cambodia Borders

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Rickettsioses have been reported in military troops deployed to military areas of operation (AOs) along northern Thai-Myanmar and Thai-Cambodia borders. Normally, rickettsiae are intracellular bacteria maintained in nature by small mammalian hosts and blood sucking arthropod vectors. To better understand how these pathogens are transmitted to humans, we investigated these AOs for the presence of rickettsial reservoir hosts and vectors. We collected arthropods from livestock, pet animals and humans in the AOs and used Rickettsia-Orientia duplex nested PCR and sequencing to detect and identify rickettsial agents. From April 2008 to March 2009, we collected a total of 133 arthropods including twelve species of fleas, lice, and ticks. We detected rickettsial genes in 80.7% and 74.8% of arthropods from Northern Thai-Myanmar and Thai-Cambodia border areas, respectively. Species of pathogenic rickettsiae identified by 17 kDa sequence analysis were Rickettsia japonica, R. rickettsii and R. massiliae. Those rickettsiae were detected in ticks (Dermacentor sp., Rhipicephalus sp. and Haemaphysalis sp.) collected from humans and pet dogs. Spotted fever group Rickettsia sp. similar to Rickettsia sp. cf 1, 5 and Rickettsia sp. SE 313 were also detected in fleas (Ctenocephalides canis, C. felis and Echidnophapga gallinacean) and lice (Liperus caponis, Menopon gallinae and Haematopinus asini) collected from dogs, cats, cattle and chickens. Orientia tsutsugamushi DNA was not detected. Using ISE6 tick cell culture, we isolated 2 rickettsia obtained from Dermacentor and Haemaphysalis ticks collected from dogs in AOs along the Thai-Cambodia border. Specific species identification of theses isolates is ongoing. These findings indicate these AOs are endemic foci for rickettsioses. This information is crucial to establish an effective disease prevention and control strategy specific to such areas.

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Failure of Artesunate plus Sulphadoxine-Pyrimethamine to suppress human infectiousness in a rural Tanzanian population exposed to intense malaria transmission.

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Objective: To evaluate the effect of supplementing Sulphadoxine-Pyrimethamine (SP) with Artesunate (AS) as the front-line malaria therapy on the infectiousness of the human population to vector mosquitoes

Design: Adult mosquito surveys were conducted longitudinally from January 2002 to September 2004 in two large-scale study sites. SP was supplemented with AS from March 2003 onwards in Rufiji District while the contiguous districts of Kilombero and Ulanga utilized SP monotherapy throughout this period.
Setting Both study sites are low-lying and predominantly rural with holoendemic Plasmodium falciparum transmission and entomological inoculation rates (EIR) exceeding 300 infectious bites per person per year.
Participants: All residents of Rufiji and Kilombero-Ulanga Districts.
Intervention Supplementation of SP with AS as the front line malaria therapy available at public-sector health facilities.
Main outcome measures: Prevalence of P. falciparum oocysts and sporozoites and transmission intensity measured as EIR.
Results: Controlling the effect of site, mosquito species, and collection period, supplementation of SP with AS was associated with increased oocyst prevalence (OR [95%CI] = 3.7 [2.8, 5.2], P < 0.001) but had no consistent effect on sporozoite prevalence (OR [95%CI] = 1.1[0.8, 1.3], P = 0.512).
Conclusion: In such settings where rapid re-infection and semi-immune, chronically infectious, asymptomatic carriers are common, ACTs have little impact on the overall infectiousness of the human population. The primary role of ACTs in reducing malaria morbidity and mortality may only accrue the secondary benefit of suppressing transmission where it is already low or can be sufficiently reduced with complementary interventions.

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PROOF OF CONCEPT: ELIMINATION OF *TAENIA SOLIUM* TAENIASIS/CYSTICERCOSIS IN NORTHERN PERU

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Infection of the human brain by the cystic larvae of the pork tapeworm *Taenia solium* (neurocysticercosis, NCC) is the most frequent cause of late onset seizures and epilepsy in the world. Endemic in most developing countries, NCC is not a rare diagnosis in the US and Europe because of increased immigration from endemic areas. While NCC is the clinical expression of human disease, transmission of *T. solium* is sustained in rural areas through a pig-human cycle where humans harbor the intestinal tapeworm and pigs carry the cystic larvae in their flesh. *Taenia solium* is claimed to be potentially eradicable, but no sustainable elimination has yet been proven. With funding from the Bill & Melinda Gates Foundation, we systematically compared the efficacy of several intervention strategies, and in a second round, tested the two most successful interventions in combination with a pig vaccine. The first round compared, in 42 villages with an estimated population of approximately 17,000 inhabitants and 6,800 pigs, six interventions: mass chemotherapy of humans and pigs (two different schemes), targeted chemotherapy, strategic treatment (treating all pigs at age three months), culling/replacement of the porcine population, and health education. Analysis of porcine incidence after the first round of interventions suggested that elimination had not been achieved. The second round selected one mass chemotherapy scheme and targeted chemotherapy which were both applied with and without a porcine vaccine (TSOL18) in a factorial design. This round was applied in 17 bigger villages with also approximately 17,000 inhabitants and 5,800 pigs. After the second intervention, all pigs were tested by antibody serology and most seropositive pigs were bought and carefully dissected. From 658 pigs including 411 seropositive (307 strongly seropositive), only 8 apparently viable cysts were recovered in 6 animals (4/8 in brain). None of these was able to evaginate when tested in vitro. In short, we demonstrated that transmission of *Taenia solium* can be interrupted in field conditions. Controlled refinements of the successful schemes are required to allow sound control in other endemic regions.

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Durable Lining (DL) Used in a Rural Village of Mali: acceptability, durability and efficacy

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Indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs) are two major large scale insecticide-based vector control strategies currently in use. IRS is efficient however geographic coverage in remote rural settlements is often limited because getting to many villages for applications can be challenging.
A trial was undertaken to evaluate acceptability and practicality of Durable Lining (DL) as an alternative vector control technology to IRS in N'Galamadibi, a village 130 km Northeast of Bamako, Mali. Deltamethrin-incorporated DL (170mg/m^2) was installed in 24 houses based upon their representation of typical rural construction materials (mud walls, thatch or metal roofs). At three, six, and nine months post-install entomological assessments of residual efficacy were conducted using WHOPES cone tests. Also a post-installation survey was made of user impressions of the DL appeal and appearance, changes in indoor environment, and impact on perception of mosquito presence.
Mortality was 97.5% - 100% in efficacy tests through 9 months post-install using susceptible *Anopheles* mosquitoes. All participants

liked the DL nine months after installation. No major defects were noted. Occupants experienced no adverse reactions. No odor was noted by any resident at nine months post installation although a slight change in odor had been reported by 37% of residents when queried three weeks after installation. The majority of participants noted no change in light or temperature in rooms where DL was installed. However, 18.2% and 27.3% respectively noted they thought there was an increase. This study reports on the acceptability to homeowners, durability of product, and efficacy/performance of DL as a potential replacement for IRS.

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New class Ib putative colonization factor antigens of ETEC from Egyptian children with diarrhea

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ETEC infections remain among the leading causes for pediatric and traveler's diarrhea. Around 50% of ETEC lack a detectable colonization factor (CF) although the majority of CF-lacking strains are LT-associated. This may be ascribed to the presence of CF not routinely included in screening panels or hitherto unidentified CF. During the last decade we have identified four new ETEC adhesins, namely PCFO39, Fim 4089, Fimb4264 and Fimb7162 that demonstrated homology to human CS12, CS18 and CS20 (class Ib) and porcine 987. We developed specific monoclonal antibodies (mAbs) for each of the new four CFs, CS18, CS20 and set of degenerate PCR primers targeting class 1b fimbriae, to screen for class Ib fimbriae. A collection of 466 phenotypically CF-negative ETEC isolates obtained from diarrheal cases as a part of a community-based study for the surveillance of diarrhea in Abu-Homos, Nile Delta were screened for the presence of CF using mAbs by dot-blot assay. PCR with degenerate primers sets, followed by DNA sequencing analysis identified novel class Ib related ETEC. ETEC candidates with novel CF were analyzed for the presence of thermo-regulated protein by SDS-PAGE and examined for adherence properties using Caco-2 assay. Phenotypically, 5.2% (n=24/466) of ETEC expressed CS20 (n=13), PCFO39 (n=3), Fimb4089 (n=3), Fimb4264 (n=2) or Fimb7162 (n=3). Genotypic characterization of the remaining CF-negative ETEC identified an additional 27 isolates related to class Ib family. Nucleotide sequence analysis of the PCR products revealed eight sequence types, two of which were identical to CS20 (n=4) and O39 (n=2) sequences that were phenotypically unexpressed. Six novel sequence types were identified: O39 like-1 (n=8), followed by O39 like-2 (n= 6), and Fim7162-like (n=4), in addition to three putative CFs with no significant similarity found with the above mentioned CFs. Surface proteins of 4 out of 6 ETEC-expressing novel sequences demonstrated a thermo-regulated proteins with MW ranging from weight ranged from 18-23 kDa by SDS-PAGE. Moreover, 3/6 strains exhibited positive adherence (≥10%) to Caco-2 monolayers (12%-100%). Data obtained suggest that the prevalence of CS20 and antigenically/genetically-related CFs in ETEC isolated from pediatric diarrhea indicates the discovery of a large fimbrial family.

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Chimeric *Plasmodium* CSP/MSP1 hybrid protein induce protection against infection and severe anemia

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We have previously reported the design of two chimeric recombinant proteins based on the circumsporozoite protein (CSP) and the Merozoite Surface Protein-1 (MSP1) derived from *Plasmodium yoelii*. Proof of principle studies using these constructs indicated that each protein used as a single immunogen induced protective immunity in mice. A distinctive feature of such chimeric proteins is the expression of promiscuous CD4+ T cell epitopes derived from the native homologous proteins. To evaluate the potential synergistic effect of combining these chimeric proteins in a single immunogen, we constructed a synthetic gene codon optimized for expression in *E. coli* that encodes both chimeric antigens associated in tandem. The hybrid chimeric protein, that contain the amino terminal chimeric CSP fused to the chimeric MSP1 protein interspaced with Gly-Pro-Gly-Pro-Gly spacers and a tag sequence, was expressed in soluble form with high yield. Antibodies produced against different segments of the recombinant protein and anti-tag sequences were used for biochemical characterization of the protein and analysis of the antigenic integrity. Groups of CAF1/J mice were used to test the immunogenicity of the hybrid construct. The immune responses elicited by immunization with the hybrid protein were compared with that obtained using a mixture of the two chimeric proteins. A single immunization with the hybrid protein induced higher antibody titers against individual component in comparison with mice that received the mixture of the two proteins. Both vaccine formulations induced robust protection to the experimental challenge with sporozoites but only the hybrid protein was able to induce sterilizing immunity. Relevantly, efficacy against experimental challenge includes protection against hyper-parasitemia and malarial anemia. The fine specificity of the immure responses induced by immunization with the chimeric hybrid protein and the effector mechanisms involved in protection will be discussed.

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Identification of malaria blood-stage inhibitors present in GSK corporate collection.

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Current antimalarial therapies target at most three or four of the parasite's metabolic pathways. Sequencing of *P. falciparum* genome has revealed more than 5000 genes and it's expected that a significant number of these genes will encode for drugable proteins essential for the intraerythrocytic stages of the parasite that could be targeted for new therapies
GlaxoSmithKline has tested the 2 million compounds present in its corporate compound library, used for HTS, in a whole-cell screening approach using the 3D7 *P. falciparum* strain. The assay uses parasite lactate dehydrogenase activity as readout for *Plasmodium* growth. This assay is non-radioactive and suitable for use in a high-density format without the need for filtration or centrifugation steps. It was carried out largely manually with excellent reproducibility, making it useful for low technology settings. We used a test concentration of 2 uM for the compounds, as preliminary experiments and past experience in the company indicated that few general toxicants are active at that concentration. A confirmed hit rate defined as more than 80% growth inhibition was 0.7%, and only 10% of the hits appeared to affect the HepG2 human cell line at 10 uM. Also most of the inhibitors demonstrated activity against the MDR Dd2 strain.
We expect that this set of compounds will be a useful source of antimalarial leads and of chemical probes for target validation. Compounds flagged as potential cytotoxics and those considered non-drug like structures have been retained, because they can still be used as tool compounds for studying the biology of the parasite. This set contains a manageable number of compounds that can be used to screen with assays not amenable to HTS. An example will be presented

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*Trypanosoma cruz*i infection of leptin deficient mice results in increased parasitemia and mortality

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Previously we reported that in the mouse model of *T. cruzi* infection (Chagas disease) hyperglycemia is detrimental to the host. The course of *T. cruzi* (Brazil strain) infection was examined in FVB-*db/db* which are hyperglycemic (baseline blood sugar 500 to 600 mg/dL), obese (60 grams) and in whom the leptin receptor is deleted. This was compared with *T. cruzi* infection in NSE-Rb *db/db* mice (transgenic). In these mice the leptin receptor is found only in neurons in the brain. They are non-obese and relatively normoglycemic. The FVB wild type mice are lean and have normal baseline blood sugars. When FVB and transgenic mice were infected with $1x10^4$ trypomastigotes there was no mortality and a low transient parasitemia. In contrast, infected *db/db* mice displayed increased parasitemia, mortality and tissue parasitism. There was a reduction in body fat percent in all infected mouse groups but the fold-decrease was greater in infected *db/db* mice. *T. cruzi* infection caused a significant reduction in blood glucose in all infected groups but the fold-decrease was greater in infected *db/db* mice. Adiponectin levels which are reduced in inflammatory states were reduced in all three infected groups but the pre-infection base-line values for the *db/db* mice were significantly lower. Plasma levels of the chemokines MCP-1, MIP-1α and RANTES and the pro-inflammatory cytokines TNF-α and IL-6 were significantly increased in infected *db/d*b mice. These data provide the first indication that *T. cruzi* infection may be controlled directly or indirectly by leptin signaling in the brain.

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Effect of aspirin in *Trypanosoma cruzi* infection and prostaglandin production.

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Trypanosoma cruzi synthesizes thromboxane A_2 (TXA_2) by a parasite TXA_2 synthase (TXA_2S). Previous experiments using TXA_2S null mice strongly suggested that 70-80% of the circulating TXA_2 in infected WT and TXA_2S null mice is parasite derived. TXA_2 receptor (TP) null mice displayed increased parasitemia, mortality and cardiac pathology compared with WT and TXA_2S null mice. We infected CD-1 mice with the Brazil strain and treated them with the cyclooxygenase (COX) inhibitor aspirin (ASA). There was increased parasitemia and mortality in this group compared with infected untreated mice. In the plasma of infected mice, the levels of TXA_2 measured as TXB_2 by ELISA were reduced in the ASA-treated mice, indicating that the parasite TXA_2 biosynthetic pathway is also inhibited by ASA. As TXA_2 production was not totally blunted, it is possible that parasite COX is not as sensitive to ASA as host COX. In order to determine if production of TXA_2 by the parasite depends on scavenging of PGH_2 from the host we infected COX-1 and TXA_2S null mice and measured the level of TXB_2 in the plasma. In both null mice types TXA_2 production was detected as early as 20 days post infection, indicating that the parasite arachidonic acid pathway is capable of generating TXA_2 independent from the host. Since the production of TXA_2 in COX-1 null mice is about half that of TXA_2S null mice, it is possible that parasites scavenge

PGH_2 from the host as one mechanism by which parasite derived TXA_2 is produced in TXA_2S null mice. We do not precisely know the role of COX-2 in *T. cruzi* infection, but we have observed that COX-1 and COX-2 protein levels remain unchanged in cardiac tissue during infection and there is no increase in COX-2 levels in COX-1 null mice by immunoblotting. The finding that the parasites can produce a vasoactive prostanoid is of immense clinical importance. A deeper understanding of the interplay of the parasite and host derived vasoactive substances in this infection may lead to the development of new strategies for drug design and treatment.

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A randomized trial of the efficacy and safety of Piperaquine in combination with DHA or SP for Seasonal Intermittent Preventive Treatment (IPT) in Senegalese children

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Introduction
In the Sahel, malaria transmission is seasonal with the disease burden limited to three months a year. Seasonal IPT has been found very effective in reducing malaria morbidity (Cisse et al., 2006).
Background
In 2006 we compared several antimalarial combinations for seasonal IPT. SP+Amodiaquine was most efficacious but presented the highest rate of adverse events (Sokhna et al., 2008). We therefore assessed the efficacy and safety of piperaquine when used for seasonal IPT.
Methods
This trial recruited all children under 5 years of age living in the responsibility zone of Keur Soce, rural Senegal, who met inclusion criteria. The intervention consisted of administration of treatment doses in September, October and November of either Dualkin® (sulfalene-pyrimethamine plus amodiaquine over 3 days) SP+AQ, Duocotexcin (piperaquine plus dihydroartemisin over 3 days) PQ+DHA, or Sulfadoxine-pyrimethamine plus piperaquine over 3 days SP+Piperaquine.
For pragmatic reasons, drugs were delivered by health post volunteers, dosage based on age and doses on days 2 and 3 unsupervised. Cumulative incidence of malaria, safety, prevalence of parasitaemia, SP-resistant mutations and anaemia in December were the major endpoints.
The trial was powered for non-inferiority in incidence of malaria (5% non-inferiority margin) and superior tolerability.
Results
Piperaquine-based combinations are as efficacious as SP+amodiaquine but better tolerated. Additionally, a very low prevalence of SP-resistant genotypes was detected with SP+Piperaquine. Combinations of two long-acting drugs are most suitable for prevention and are highly effective against emergence of resistant parasite genotypes. Importantly, this approach allows ACT to be reserved to treat clinical malaria.

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Role of innate immunity in regulation of Wolbachia infection level in Aedes aegypti

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Infections by mosquito borne dengue viruses lead to serious disease in many tropical areas of the world, causing illness, death, and a large amount of economic injury to those affected. Currently, there is no vaccine or specific treatment for the disease, so control of the mosquito vector population is the primary intervention tool. Proposed genetic control strategies include using the intracellular bacterium *Wolbachia* as either a population suppressant or a gene driver to spread the traits required to reduce mosquito vectorial capacity for dengue viruses. These strategies require a better understanding of the interactions between *Wolbachia* and its mosquito host, including potential natural mechanisms for the removal of *Wolbachia* from its hosts. In *Ae. aegypti*, the presence of *Wolbachia* was observed to induce the expression of a number of innate immune genes. To investigate whether mosquito innate immunity plays any role in the regulation of *Wolbachia* infection levels, we employed RNA interference and genetic engineering to manipulate mosquito immunity, and challenged mosquitoes with various pathogens. Mosquito immune pathways were observed to regulate the density of *Wolbachia* in both the mosquito ovaries and remaining carcass tissues. Our results provide a novel insight into the ecological relationships between the mosquito and its intracellular parasites. We discuss our findings in relation to the future use of *Wolbachia* to block dengue transmission by mosquitoes.

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Insulin Receptor and Target of Rapamycin in autogenous Culex tarsalis

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Populations of medically important mosquitoes may display various reproductive strategies. While most mosquitoes are anautogenous, requiring a blood meal in order to reproduce, autogenous individuals are able to mature an initial batch of eggs in the absence of a blood meal. Both natural and laboratory populations of the arboviral vector, *Culex tarsalis*, show varying degrees of autogeny due to a combination of genetic and environmental factors. Recent studies have demonstrated the importance of both the Insulin and Target of Rapamycin pathways in blood meal regulated vitellogenesis and egg development. To investigate the function of these pathways in autogeny, we first selected for and characterized primarily autogenous and anautogenous populations of *Cx. tarsalis*. We then cloned portions of the *Cx. tarsalis* Insulin Receptor and Target of Rapamycin mRNA and, using our selected lines, studied the expression and function of these pathways and autogenous ovarian development.

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Modeling the potential spread of yellow fever by infected air travelers

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Yellow fever virus is a highly pathogenic virus with epidemic potential in human populations. It has largely been controlled in human populations by vaccination and vector control, but continually reemerges due to persistence in sylvatic transmission cycles. Though reemergence tends to occur in remote areas, modern human movement patterns may result in rapid translocation of infected individuals to major urban centers where there is risk of urban transmission vectored by *Aedes aegypti* mosquitoes. We developed a model to simulate potential global spread of yellow fever virus via infected air travelers given introduction into a single large urban area with strong connections to the global airline network. The cities selected and modeled were major international airline hubs and/or cities close to areas where yellow fever emergence is a risk. Each city was modeled with climate dependent *Ae. aegypti* populations and human populations with subpopulations in various states relative to yellow fever infection: susceptible, incubating, and infectious for the vectors, and susceptible, incubating, infectious, and immune for humans. In the model, human travel between cities was simulated based on actual air transportation data. The human populations in each city are then allowed to travel according to a model based on actual air travel data. When incubating travelers reach another city, they may become infectious and begin a transmission chain there. In constructing the model, we paid special attention to proper treatment of infectious periods and travel characteristics and performed significant sensitivity analysis. The result of this work is a stochastic model that can be used under different scenarios and assumptions to assess the probability of the international dissemination of yellow fever by infected travelers.

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Classification of dengue illness based on readily available laboratory data

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Background: Controversy surrounds current WHO guidelines for the diagnosis of DHF, in part due to the need for convalescent hematocrit or X-ray to detect pleural effusion. The aim of this study was to examine the sensitivity and specificity of dengue illness classification based on clinical laboratory data from only during the acute illness. Methods: We analyzed data from a prospective study of Thai children aged 6 months to 14 years who presented to Queen Sirikit National Institute for Child Health (QSNICH) or Kamphaeng Phet Provincial Hospital (KPPPH) with fever onset <72 hours and temperature≥38°C prior to entry. Clinical laboratory data were collected daily. Variables used included maximum values of AST, ALT, hematocrit, % neutrophils, % lymphocytes, and % monocytes, and minimum values for platelets and leukocytes. Multivariable logistic regression models were established based on variables yielding the highest area under the curve from univariate analyses. These models were used to determine a probability cutoff that best distinguished: 1) DHF vs. DF, 2) DHF vs. DF + other febrile illness (OFI), 3) Dengue vs. OFI, 4) Severe dengue vs. non-severe dengue + OFI. Data from QSNICH were used as a training dataset and each model was validated using KPPPH data. Results: A total of 1227 (QSNICH n=1058, KPPPH n=169) patients were included in the analysis (228 DHF, 386 DF, and 613 OFI). The sensitivity of the models ranged from 89.2% (dengue vs. OFI) to 79.6% (DHF vs. DF). When each model was applied to the validation set, the sensitivity decreased slightly but remained high. Conclusion: We established logistic regression models to classify disease severity in patients with dengue without relying on X-ray, ultrasound, or calculation of percent hemoconcentration. The models demonstrated high sensitivity in the validation dataset from a different hospital and catchment area. These models could be used to calculate a probability risk of DHF or severe dengue based on readily available clinical laboratory data and will need to be validated in other dengue endemic regions.

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Secreted microfilarial products induce human monocytes with phenotypic and functional characteristics of alternatively activated macrophages

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Among the many mechanisms proposed to mediate the profound filarial-specific T cell hyporesponsiveness seen in lymphatic filariasis, the two given most credence are an IL-10- mediated regulation and/or a defect in antigen presenting cell (APC) function. The latter concept is based on findings showing that monocytes from patients with patent filarial infections are both studded with internalized filarial antigens and express markers associated with alternative activation of macrophages such as resistin, arginase-1, and CCL18.
To further explore the role of APCs in filarial infection, and to determine whether exposure of monocytes to live microfilariae (mf) changes the characteristics of these cells, purified human monocytes were exposed to mf of *Brugia malayi* and phenotypic markers and cell function were compared to those of monocytes exposed to factors known to generate either alternatively activated macrophages (IL-4) or classically activated cells (MCSF). Similar to IL-4, live mf induced significant upregulation of Fizz3, CCL17, CCL18, CCL22 as compared to mf-unexposed monocytes ($p=0.01$ to 0.04). Furthermore, similar to IL-4, mf downregulated the cell surface expression of HLA-DR. Interestingly, while IL-4 upregulated the cell surface expression of PDL1, PDL2, CD206, and CD86 on human monocytes, soluble factors from mf significantly downregulated the expression of PDL2 and CD86 ($p=0.03$) on these cells. Moreover, exposure of monocytes to soluble factors from mf significantly downregulated the gene expression of TLR3, TLR4, TLR5, and TLR6, and TLR7 in human monocytes ($p=0.01$ to 0.04) and resulted in decreased production of IL-10 and MIP-1α in response to TLR4 ligand. Finally, in contrast to MCSF cultured monocytes, exposure of monocytes to mf or IL-4 resulted in significant inhibition of phagocytic ability of these cells.
In Summary, our data suggest that despite significant similarities in the phenotype and function of monocytes exposed to IL-4 and mf, mf also have unique effects on the expression of TLRs and costimulatory molecules on monocytes. The contribution of these effects to the observed filarial-specific T cell hyporesponsiveness in infected patients remains to be elucidated.

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Derivation and characterization of a *Leishmania major* axenic amastigote-like population for high throughput screening

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Leishmania spp. exist in promastigote and tissue-based amastigote life cycle forms. Promastigotes are ideally suited for high throughput screening (HTS) drug discovery efforts because they are easy to manipulate and are amenable to multiple HTS assay formats. However, tissue-based amastigotes are considered by some to be the most relevant form of the parasite as they are the form found in mammalian hosts. Tissue-based amastigotes are morphologically and biochemically distinct from the promastigotes, but they represent a more challenging experimental system since they require the host cell milieu. Thus, tissue-based amastigotes are often less amenable to HTS adaptation, validation and implementation. An alternative experimental approach is the generation of "axenic" amastigotes from promastigote populations using a combination of temperature and pH manipulations. We systematically derived a *Leishmania major* axenic amastigote-like population (pH 4.9, 32^{o}C) for use in anti-leishmanial HTS drug discovery efforts. Our axenic amastigote-like population shares many characteristics of tissue-based amastigotes including a general rounded morphology, lack of flagella, extended parasite doubling time (~24 hours) and differences in intracellular protease activity profiles versus promastigotes. We used this *Leishmania major* axenic amastigote-like population to evaluate the growth inhibitory activity of 47 experimental compounds as well as 12 compounds of known pharmacological action that exhibited submicromolar growth activity in a promastigote drug susceptibility assay. The majority of compounds maintained growth inhibitory activity albeit at lowered potency (*i.e.*, micromolar). Importantly, multiple compounds maintained nanomolar growth inhibitory activity under these new parasite culturing conditions. Thus, this axenic amastigote-like population represents a valuable research tool to help evaluate potential anti-leishmanial compounds for efficacy and stability in a low pH environment.

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Post-integration stability of PiggyBac transposable element in Aedes aegypti cell line

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PiggyBac transposable element has been widely used as a gene vector in mosquitoes but shows no evidence of post-integration mobility in *Aedes aegypti* mosquito that greatly limits its use as a functional genomic tool and gene drive vector in the genetic modification of *A. aegypti*. A transgenic *A.aegypti* cell line with a single *piggyBac* element was created and the post-integration mobility of the element was analyzed. The *A. aegypti* cell line was co-transfected with plasmids carrying *piggyBac* element with a

hygromycin resistance gene and an EGFP marker gene driven by *Drosophila Actin5c* and viral *ie1* promoters, respectively. Initially 16 clones were positively selected for hygromycin antibiotic resistance and 2 (cell line 5 and 8) were successfully established as transgenic cell lines. Analysis of the genome of cell line 8 demonstrated the presence of a single *piggyBac* element. Inverse PCR and subsequent amplification of the junction DNA fragments containing the left and right terminal inverted repeats of *piggyBac* elements with mosquito genomic DNA confirmed the element integrated was transposase mediated. Cell line 8 was transfected with a plasmid containing *piggyBac* transposase gene and DsRed marker gene driven by *hsp70* and *polyubiquitin* promoters from *D. melanogaster*, respectively. As a control, cell line 8 was transfected with a control plasmid lacking the transposase gene. 24hrs after transfection, the cells were heat shocked (37^{o}C/1hr) to induce transposase expression. Expression of functional *piggyBac* transposase in heat-shocked cells was confirmed using a plasmid-based excision assay. Transfected cells were sorted and genomic DNA from cells expressing EGFP and DsRed was analyzed by TE display to look for *piggyBac* remobilization. The analysis revealed no evidence of remobilization of the *piggyBac* element. Earlier studies in our laboratory showed the post-integration stability of the *piggyBac* element in the genome of *A. aegypti* mosquito. These experiments show that the post-integration behavior of *piggyBac* element in *Aedes* cell line is the same as observed in the soma and germline of the adult mosquitoes. This cell line could serve as a good model to study the post-integration behavior of the transposable elements. This cell line system is being used to explore the factors responsible for repressed element mobility in this species.

3086

In vivo West Nile virus fitness determination by quantitative sequencing

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Genetic variation affects West Nile Virus (WNV) replication, pathogenesis and transmission dynamics. To assess genetic correlates of WNV fitness, we constructed a genetically marked “control” virus for use in competitive fitness studies, assessed various methods for determining the relative proportion of test and control viruses in mixed infections, and measured viral fitness *in vivo* in mosquitoes and chickens. To generate the control virus, a string of five noncoding changes were inserted into nucleotide positions 8313-8317 of a WNV cDNA clone by PCR mediated mutagenesis such that the parental sequence CTC TCA CGG was changed to CTa agc aGG. To determine the best method of quantifying the relative proportion of wild-type (WT) and control (REF) virus, RNA from each genotype was diluted and mixed 1:9-9:1, and 3 quantification methods were compared. The first method was a TaqMan assay using genotype-specific probes. The second method used molecular clones of PCR-amplified viral RNA and a subsequent TaqMan assay with genotype-specific probes (above) to determine the genotype of each colony. The third method, quantitative sequencing, uses a sequence chromatogram to quantify the area under the trace curves corresponding to each genotype. Molecular cloning detected 12.5% of the “REF” genotype when the true proportion was 10%, resulting in the most accurate quantification of the three methods. However, this method would require testing at least 50 clones from each mosquito tissue in order to reliably detect small percentages of viral RNA. Both quantitative sequencing and TaqMan detected near theoretical values when the mixtures are within a 60-40% range, but quantitative sequencing clearly outperformed TaqMan as the values became more extreme. When REF:WT were mixed 20:80% the TaqMan assay detected 0.8% of Ref and 99% of WT, while quantitative sequencing detected 14.5% of REF and 85.5% of WT. Therefore, we used quantitative sequencing to assess WNV fitness *in vivo* in mosquitoes and chickens.

3087

Evidence of occupational exposure to Hantavirus and other vector-borne viral pathogens among mammalogist and other field workers in Peru

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Hantavirus cases have been reported in increasing frequency from many South American countries since the discovery of Sin nombre virus in the US in 1993; however, no human cases have been identified from Peru. In 1999, a hantavirus was isolated from a pigmy rice rat in the city of Iquitos, Peru. Despite this finding, there have been no efforts to identify infection with these viruses among those with direct occupational exposure to rodent species - the primary reservoir hosts for hantaviruses. We conducted a serological survey and risk factor study among mammalogists and other field biologists to determine if individuals in this population had any evidence of antibodies to hantaviruses and to identify any specific risk factors for infection (i.e., lack of personal protective equipment use, etc.). This study was conducted among 166 participants, all of whom completed a self-administered risk factor questionnaire. Additionally, we collected a blood sample to test for evidence of IgG specific antibodies to these viruses using ELISA. Among the participants, 57.2% were male; the mean age was 29±9.6 years; 5.8% were field biologists, 7.7% ecologists, 16.1% mammalogists, and 70.3% were other field workers, mostly veterinarians and biology students. Additionally, 66.2% performed field work involving small mammals directly. We identified an antibody prevalence of 0.7% (1/136) to New World hantaviruses (NWH; Sin nombre virus antigen) and 1.5% (2/136) of antibodies to Old World hantaviruses (OWH; Seoul virus antigen). The one NWH positive individual referred to working in different locations throughout Peru and other South American countries, while the two OWH positive cases reported to have worked only in Loreto (north-eastern rainforest area; location of Iquitos) or Lima. Our findings suggest that human infections of

hantavirus do occur in Peru and that field workers may be at risk of infection due to the nature of their work. These high-risk populations could likely benefit from training programs and educational materials on ways to help the minimize risk of exposure to rodent- and other vector-borne diseases.

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Characterization of Human Innate Immunity to Rift Valley fever virus

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Rift Valley fever virus (RVFV), the causative agent of Rift Valley fever, is transmitted endemically in sub-Saharan Africa by a number of mosquito species, and causes periodic epidemics during times of high rainfall. Due to the potential for dispersion to new areas, and the significant threat to human health and agricultural interest, RVFV is listed as a Category A select agent. Clinical manifestations in humans may consist of hemorrhagic fever, encephalitis, and retinitis. While attenuated RVFV strains (i.e., MP-12 and Clone 13) have been studied as vaccine candidates, neither the pathogenic mechanisms of virulent RVFV nor the protective mechanisms of attenuated RVFV are fully understood. Thus, it is critical to investigate how host innate immunity is involved during RVFV infection in order to better understand factors contributing to pathogenesis. We employed cDNA microarray-based functional genomics to compare global gene expression responses elicited by two different strains of recombinant (r), attenuated RVFV, i.e., rMP-12 and clone-13-like rMP-12 infected primary human macrophages. Preliminary findings indicate primary human macrophages are highly permissive to RVFV infection. Furthermore, RVFV carrying the virulence factor and IFN antagonist, non-structural S (NSs), dampens a broad array of host innate antiviral responses, including but not limited to previously identified interferon related molecules; a phenomenon demonstrated by dramatically enhanced gene expression of a broad range of antiviral factors after infection with NSs-depleted clone 13-like rMP-12, in comparison to those infected with NSs-intact rMP-12. In addition we observed the surprising finding that macrophage phagocytic function, measured by dextran-sulfate uptake, was reduced after infection with RVFV, and this activity decreased more dramatically in NSs deleted clone-13-like infected macrophages. Ongoing research includes further analysis on the involvement of transcription factors and host defense signaling pathways during RVFV infection.

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Malaria is Declining but Fevers are Not: Investigating Etiologies of Fevers of Unknown Origin

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Background: Fevers of unknown origin (FUO) are common presentation to health care facilities in Kenya. Patients presenting with FUO are often empirically treated for malaria. Though understandable in some circumstances, this practice leads to inadequate treatment, inaccurate disease reporting, and late recognition of emerging infections, outbreaks and epidemics.
Methods: Patients (n=576) presenting at a network of clinical sites in Western Kenya were recruited for the study. Cases of malaria were screened out using rapid diagnostic tests. Nucleic acids were isolated from nasal samples and whole blood and evaluated by RT-qPCR for: malaria, dengue, rickettsia, brucellosis, leptospirosis, salmonellosis, measles, and upper respiratory tract viruses. Arbovirology cultures for yellow fever, alphaviruses, arenaviruses, bunyaviruses, filoviruses, flaviviruses, and phleboviruses, Ebola, Lassa, and Marburg were performed at KEMRI Arbovirus laboratory.
Results: Of the 576 patients, 150 were evaluated for the multiple etiologies. Rickettsia infections were identified in 2.4% of children. Despite screening malaria out, 4.7% of the patients were found to have malaria by RT-qPCR. On speciation, all have been identified as *P. ovale*. A very high prevalence of *Salmonella spp* (38%) were identified in the blood of children recruited. Of the viral respiratory tract infections, 50% have been flu B, 10% flu A, 8% rhino virus, 7% adeno, 8% parainfluenza, 12% RSV A and 2% Corona virus OC 43. Many patients did not have identifiable etiology even after culture.
Conclusion: This study is a valuable attempt to catalogue etiologies of FUO in the area of study and will increase our understanding of the diseases, where they are likely to occur, at what frequency, and will result in improved treatment, reporting and recognition of emerging and re-emerging diseases. Of note is the malaria failure rate of 5% when RDTs are used as diagnostic tools. As surveillance expands to cover other ecological sites, diverse etiologies of fever are likely to be identified.

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Regional phylogenetic structure of *Trypanosoma cruzi* vectors inferred from geographical co-distributions networks

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Regional phylogenetic structure of *Trypanosoma cruzi* vectors inferred from geographical co-distributions networks
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A major concern in evolutionary ecology and biogeography is the study of species distribution, especially when species from the same genus have a phylogenetic structure showing non-random association. This pattern can emerge when traits and niche conservatism occur. In species with public health importance, such as insect vectors, looking into these patterns is of great relevance since processes keeping or avoiding the phylogenetic structure can be key factors in developing control and prevention measures. Here we use a novel statistical method to analyze species dependence in the Triatominae (Reduviidae) based on collection data points of the main Chagas vector's in Mexico. A metric (Epsilon) for codependence in specie's distribution was built and compared to a null model of random distribution. Finally, using linear regression we analyzed if the phylogenetic distance has an effect in epsilon values. We found species dependence ranging from negative (repulsion) to positive values (attraction).
Additionally, we found that vector's phylogenetic structure at regional scale can be related to their epidemiological importance.

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Transduction of Schistosoma mansoni with vesicular stomatitis virus glycoprotein pseudotyped lentivirus

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Retrovirus-mediated transduction offers a potential means to insert reporter transgenes into the schistosome genome, to elucidate schistosome gene function and expression through vector-based RNA interference, to establish transgenic lines of schistosomes. Previously we have reported that murine leukemia virus (MLV) pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) can transduce several developmental stages of *Schistosoma mansoni*. In addition to MLV, we have been investigating whether human immunodeficiency virus (HIV-1) lentivirus (a complex retrovirus) might be utilized for transgenesis of schistosomes. We have constructed a panel of lentiviral vectors using the ViraPower Gateway (Invitrogen) system. We modified pLenti6/R4R2/V5-DEST by insertion of an endogenous schistosome gene promoter; from the spliced leader (SL) RNA gene, upstream of the reporter gene encoding jellyfish enhanced green fluorescent protein (GFP). 293 FT producer cells were transformed with this construct and viral packaging plasmids to produce replication incompetent lentivirus virions pseudotyped with the VSVG envelope. Here we investigated early events in transduction of the schistosome tegument by the pseudotyped lentivirus. Virions were added to cultures of schistosomes in the presence of the cationic polymer polybrene. At several time points from 0 minutes to four hours thereafter, schistosomes were washed and cross-linked. Using a VSVG specific antibody as the probe, time course dependent immunolocalization was evident to both schistosomules and adult worms, with increasing fluorescence signals from 30 minutes to 3 hours after exposure. These findings indicated that time dependent fusion of the VSVG-lentivirus to schistosome surface was taking place. We are now investigating downstream events including exposure of the lentiviral capsid proteins, integration of the proviral transgenes into schistosome chromosomes, and reporter gene activity, with the aim of establishing the potential of VSVG-HIV-1 lentivirus as a vector for genetic manipulation of schistosomes.

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Worldwide population structure and the evolution of human association in Aedes aegypti

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Aedes aegypti is a human commensal mosquito that has invaded much of the tropical and subtropical world over the past few centuries. As the principle vector of both dengue fever and yellow fever, this species is enormously important from a public health standpoint. Though *Aedes aegypti* is often treated as a homogenous species in its role as a disease vector, in reality, the species displays vast morphological and ecological heterogeneity. The two described subspecies of *Aedes aegypti* (*Aedes aegypti aegypti* and *Aedes aegypti formosus*) differ markedly in their association with human habitats, as well as in their ability to transmit dengue viruses. We are currently using 15 microsatellite markers and two mitochondrial genes to describe the worldwide population genetics of *Aedes aegypti* and explore the evolution of human association in this species. Our results suggest that the African sylvan subspecies, *Aedes aegypti formosus*, is indeed ancestral to the worldwide domestic form of the species, but that close human association has likely evolved multiple times independently. In addition, a traditional two-form view of the species appears inadequate at explaining existing variation. Our analyses detect at least three major forms of the species, with additional distinct populations within forms. As we have only examined a fraction of existing *Aedes aegypti* populations, more major and minor variants remain likely to be discovered. Temporal data is available for a subset of geographic locations, and we have found that populations remain genetically stable over a period of at least three years and potentially much longer. In the future, population assignment tests will be combined with information about vector competence for dengue viruses to determine the population of origin and public health significance of new *Aedes aegypti* introductions.

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Re-emergence of Chikungunya (CHIK) in Thailand with African Strain Virus, 2008-2009

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Chikungunya (CHIK) is now a serious public health concern in South-East Asia. In 1958, CHIK virus (Asian strain) was first identified in Bangkok and subsequently disappeared in 1996. On October, 2008, a confirmed CHIK was introduced to Thai-Malaysian border provinces. Thus a CHIK case definition and laboratory protocol was developed to support national surveillance and containment activities. Case information from 2008-2009 found in the national notifiable disease surveillance system was analyzed(Epi Info, US CDC).The results of CHIK and Dengue testing performed by the Thai National Institute of Health (NIH) and USAMC-AFRIMS were also analyzed. A total of 44,040 suspected CHIK cases were reported between 2008-2009 (2,494 and 41,546, respectively). No deaths were reported. The majority of cases were reported from the four southernmost Thai-Malaysian border provinces (2008 - 99.7%, 2009- 60.0%). In 2009, CHIK spread to all upper southern provinces (38.3% of all cases reported) and included some Western and Eastern provinces (1.0%). The male to female ratio was 1:1.5. Overall, most cases were in adults (83.0%) with a median age of 34 years (range -3 mo, 96 yr). But the median age in 2009 was significantly lower than 2008 (33 and 38 yr, respectively; $p<0.001$). The proportion of child (age<15 yr) and student cases increased from 12.1% and 10.9% in 2008 to 17.3% and 18.4% in 2009 ($p<0.001$). Working in agricultural industry appeared to be a risk factor for infection in 2008 (40.0% of cases) but this trend declined in 2009 (46.1% vs 39.6%; $p<0.001$). Outpatient cases accounted for 90.6% of all cases. In total, 1,218 suspected cases were reported by NIH; 440 cases were laboratory confirmed (36%) by either RT-PCR or seroconversion by HI. The yield of RT-PCR and HI were 49.7% (388/781) and 35.3% (89/252), respectively. A suspected CHIK case return to be dengue infection was 3.9% and 1.4% was co-infected with CHIK. Molecular sequencing of CHIK virus isolates was performed by USAMC-AFRIMS and revealed historic Asian strain replacement with East/Central African Strain. CHIK continues to spread in a northward distribution throughout Thailand. The epidemic pattern in 2009 is changing from rural to urban settings as evidence by increasing infection rates among students. Continued clinical, serologic, and molecular surveillance and characterization of Thailand's CHIK outbreak is required and ongoing.

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Travel Associated Dengue Surveillance, US 2006-2008

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Objective: To describe all reported presumptive dengue cases among individuals residing in the 50 United States.
Methods: A descriptive analysis was performed using data from the Centers for Disease Control and Prevention (CDC) ArboNET, and CDC Dengue Branch and Puerto Rico Department of Health passive dengue surveillance system (PDSS) from January 1, 2006 through December 31, 2008. ArboNET is an arboviral surveillance system that receives reports of laboratory-positive dengue cases directly from state and local health agencies. The PDSS receives physician requests for dengue testing of samples from presumptive cases (those with clinical presentation consistent with dengue) residing in the 50 United States and its territories. Both systems collect clinical and epidemiologic data. Laboratory confirmation is based on (*i*) dengue virus isolation or detection by PCR, (*ii*) IgM seroconversion, or (*iii*) positive IgM in a single sample.
Results: A total of 1,126 presumptive cases of dengue were reported in 2006-08 among individuals residing in the 50 United States, a 147% increase compared to 2003-05 when 455 cases were reported. All were travel-associated (i.e., history of recent travel to dengue endemic area) and 732 (65%) were laboratory-positive. Of the 596 laboratory-positive cases reported to ArboNET, 292 (49%) were hospitalized and no fatalities were reported. Of the 530 presumptive cases reported to the PDSS, 62 (12%) were hospitalized, 136 (26%) were lab-positive, and no fatalities were reported. Only 57 cases were reported to both systems.
Conclusion: Dengue is an increasingly common disease among US residents traveling to endemic areas. However, the epidemiology of dengue among US travelers remains incompletely described. As dengue became a nationally notifiable condition in 2009, case detection and reporting may improve allowing for a better estimate of the true burden of disease.

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The Toll pathway is a conserved immune defense active against different dengue serotypes and present in multiple *Aedes aegypti* strains.

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Dengue virus has become one of the most important arboviral pathogens affecting tropical and subtropical regions of the world. Dengue virus is transmitted by the mosquito *Aedes aegypti* and *Aedes albopictus* and its transmission and disease dynamics are exacerbated by the existence of four closely related dengue serotypes. Mosquito vectors can limit infection with certain pathogens by mounting a range of immune responses. We have previously demonstrated the importance of the Toll pathway as part of the anti-dengue defense repertoire at 7 days after ingestion of an infected blood meal. In this study we have examined the activity of this immune pathway against different dengue virus serotypes at the early stages of infection in laboratory and field-derived mosquito strains. Our studies show the implication of the Toll signaling pathway in the anti-dengue defense at 72 hours post-infection. Furthermore, this immune defense repertoire is active against different dengue virus serotypes and observed in different *Ae. aegypti* strains.

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The Role of Polymorphisms in PfRH5 in Species-Specific *Plasmodium falciparum* Erythrocyte Invasion

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The owl monkey, *Aotus nancymaae,* is widely used in malaria research, but only some *Plasmodium falciparum* parasites are virulent to these monkeys. In many cases, parasites cannot infect owl monkeys because they are unable to invade their erythrocytes. Previously, we identified a key pathway for *Aotus* erythrocyte invasion in the 7G8 × GB4 cross that was determined by an isoleucine to lysine change at position 204 of the erythrocyte binding protein PfRH5. However, lysine-204 could not be the only requirement for *Aotus* erythrocyte invasion as other *Aotus* virulent *P. falciparum* clones lacked this particular residue, but carried additional PfRH5 amino acid polymorphisms. Using allelic replacement methods, we have investigated whether these amino acid substitutions also confer invasion into *Aotus* erythrocytes. Specific mutations were introduced at codons 204, 347, 358, 362, 410 and 429 of the endogenous *pfrh5* gene in avirulent 7G8 parasites. *Aotus* erythrocyte invasion rates of these transformants show robust invasion rates by 7G8 transformants carrying the polymorphisms of virulent parasites, whereas parasites expressing *prfh5* alleles derived from avirulent parasites show little or no invasion. These studies support a critical role for PfRH5 in *Aotus* infection and provide further insights into the PfRH5-dependent invasion pathway.

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Draft Genome Assembly of the Asian Malaria Mosquito, Anopheles stephensi

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We report an ongoing effort to sequence the Indian strain of the Asian malaria mosquito, Anopheles stephensi. A draft assembly has been produced based on ~1800 Mbp 454 Titanium shotgun sequences (~8 x coverage) that are 300-500 bp in length. The assembly now covers 202 Mbp of the 240 Mbp genome and 18105 genes are predicted. In addition to the shotgun sequences, we have now obtained mate-pair sequences with 1.5 kb and 20 kb inserts respectively, which will greatly improve the assembly. We also obtained transcriptome sequencing results using illumina, which facilitates the identification of transcripts. We will report the most updated genome assembly and annotation as well as a systematic comparison to the An. gambiae genome.

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Transcriptomic Comparison of Virulent and Vaccine Strain Junin Viruses

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Junin virus (JUNV) is the causative agent of Argentine hemorrhagic fever and a member of the Arenaviridae, a family which includes several important human pathogens including Machupo virus and Lassa virus. All of these viruses are NIAID category A priority pathogens and treatment is limited to supportive care. An effective vaccine for JUNV, Candid-1, is used in endemic areas. Comparison between attenuated strains, which induce protective immune responses, and virulent strains can identify targets for novel

anti-viral therapies and for rational attenuation for vaccine design. We have used mRNA microarrays and pathway analysis to investigate the responses of monocytes infected with JUNV or Candid-1. This approach has allowed us to understand the cellular responses that are associated with an appropriate immune response and viral clearance, which of these events are suppressed in virulent infection to allow the virus to evade the immune response, and which may be activated and lead to pathogenesis. Using pathway analysis, we can identify the upstream signaling events which led to these transcriptional changes and which may be modulated as a therapeutic strategy. Using k-means clustering, we have identified groups of genes which are unique and common between JUNV and Candid-1 infection. The majority of changes were associated with cell cycle, immune responses and post-translational modification. Pathway analysis identified cell-signaling pathways based-around NF-kB and Jak/STAT signaling. By relating these signaling pathways and transcriptional changes back to clinical disease, we are beginning to determine the molecular basis of pathogenesis, refine targets for continued investigation and identify potential targets for novel antiviral therapies.

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Plasmodium falciparum Liver Stage Antigen Discovery

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An effective vaccine targeting the pre-erythrocytic (PE) stages of *Plasmodium falciparum* would substantially reduce the worldwide malaria burden. Immunization with radiation-attenuated *P. falciparum* sporozoites can protect humans upon experimental challenge, highlighting the possibility of a broadly effective PE vaccine. However, gene expression profiles of the asymptomatic liver stages of *P. falciparum,* particularly the early stages, are not well defined. Therefore, we sought to define the antigens expressed by the parasite during the PE stages. We used tiling microarrays to measure gene expression in salivary gland sporozoites as well as in axenically cultured liver stages (sporozoites cultured for 24 hours in the absence of host cells). For comparison, we analyzed gene expression in blood stage parasites. In parallel, we performed RNA sequencing of 24 hour *in vitro*-infected HC04 hepatocytes, and compared gene expression in the two liver stage forms. We found a high degree of similarity in gene expression between the axenically cultured liver stages and the *in vitro*-infected liver stages despite differences inherent in both techniques. Additionally, we validated the liver stage expression level of over 100 genes by qPCR. Comparing both liver stage data sets to the blood stages, we assessed basic metabolic functions in the early liver stage parasite and found a strong similarity between pathways expressed in liver and blood stage parasites. Measuring antibody levels against some of these liver stage proteins, we found responses in a subset of Tanzanian children. In addition, we are performing IFN-g Elispot assays to measure T-cell responses against these proteins in the same children. In summary, our findings reveal the expression of novel genes during liver stage *P. falciparum* infection. Additionally, we show that some of the PE proteins identified by functional genomic approaches are recognized by sera from naturally-exposed children, calling for further work to assess them as potential vaccine candidates.

3100

Differential lethality of the Culex pipiens pipiens midgut for filarial worms

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Culex pipiens complex mosquitoes thrive in temperate and tropical regions worldwide, and act as efficient vectors of Bancroftian lymphatic filariasis (LF) in Asia, Africa, the West Indies, South America, and Micronesia. Neither Cx. pipiens pipiens (Cpp) nor Cx. pipiens quinquefasciatus, however, vector South Asian brugian LF (caused by Brugia malayi), despite their presence in endemic areas. The IA strain of Cpp is differentially susceptible to filarial worms, in that it efficient vectors W. bancrofti but is refractory to Brugia parasites. We report that the barrier to Brugia infectivity in Cpp Iowa strain is the mosquito midgut, and that the damage inflicted to incoming Brugia mf is somatic and lethal in nature. Less than half of experimentally infected Cpp were infected with B. pahangi, with infection intensities significantly lower than for the susceptible Aedes aegypti black-eyed Liverpool (LVP) strain ($p<3 \times 10^{-4}$). The Cpp strain was highly refractory to B. malayi, with prevalence of 0-5%, compared to 100% prevalence in LVP controls. Brugia mf introduced intrathoracically into Cpp developed equally well as in LVP controls, indicating that Cpp is physiologically compatible for infection. Mf isolated from Cpp midguts exhibited kinky motility, and unlike blood-derived mf and LVP midgut-derived controls, failed to develop when inoculated intrathoracically into the susceptible LVP strain. Together these data strongly support the role of the midgut as the primary infection barrier of Cpp for Brugia spp. Vital staining of Cpp midgut-derived mf occurs predominately within the body, and application of papain to midgut-derived worms removed the sheaths from blood and LVP-derived mf, but completely dissolved Cpp-derived mf, supporting the hypothesis that Cpp midgut-inflicted damage is somatic in nature. Incubation of Brugia mf with extracts of Cpp midguts produce similar phenotypes; clearly indicating that the Cpp midgut factors that damage mf in vivo are soluble and stable in physiological buffer (saline), and they are capable of attacking mf in vitro as effective as that in vivo.

3101

Use and characterization of Plasmodium falciparum invasive merozoites

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Plasmodium merozoites are the primary targets of the existing malaria erythrocytic-stage vaccine efforts. Growth inhibition studies using antibodies against merozoite invasion-related proteins such as AMA1, MSP1, EBL, RH family proteins etc., suggest a role for these proteins in invasion. How and at what step these proteins function during invasion is not clear due to the very short invasion span of purified P.falciparum merozoites. In contrast, invasive merozoites have been obtained from the simian malaria P.knowlesi. The basis for this difference in merozoite viability and invasiveness between P. falciparum and P. knowlesi is not known. Generating invasive P.falciparum merozoites will not only aid in better understanding the process of RBC invasion but also help improve development of a blood stage malaria vaccine. Invasive merozoites previously generated by gamma irradiation of parasitized erythrocytes followed by selection under low-hematocrit suspension culture for five months were used in this study. Here we report our advances in using such “long-lived” merozoites to study RBC invasion and our approaches to identifying factor(s) crucial for maintaining invasiveness. Schizont-free merozoites purified from this selected line retained invasiveness for at least 30min at RT. In addition, these merozoites could be concentrated with minimal loss of invasiveness resulting in as high as 40% rings. To study the molecules involved in invasion (to enrich for merozoites in the act of invasion), we have developed a method involving rapid fixation of merozoite invading cells followed by flow sorting, allowing us to precisely localize proteins to the moving junction. Finally, we have taken a whole cell, label-free proteomics approach using a next generation Xevo Q-Tof MS system to study quantitative and qualitative differences between merozoites from the parent and selected line. Preliminary data analyzing differences in protein expression and abundance between the two populations will be discussed.

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Improved Methods for Detection and Cryopreservation of *Plasmodium falciparum*

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Microscopy remains the gold standard for diagnostic confirmation of malaria, while RDTs and PCR based methods are increasingly employed for field and lab applications, each with limitations. Cryopreservation methods commonly employed successfully preserve only early ring stage asexual parasites. We are working to develop improved methods for PCR-based detection and cryopreservation of multiple developmental stages of *falciparum* malaria. Using *in vitro* culture-adapted *P. falciparum* parasite isolates, we analyzed several methods to prepare samples for PCR typing. Parasite-infected erythrocytes were subjected to hypotonic lysis, followed by a near complete depletion of the trace amount of hemozoin and hemoglobin, resulting in an improved detection limit of one parasite in 1x 10^9 - 10^{10} RBCs. We analyzed targets, including *msp2-*, *msp1-*, *SSUrDNA-* and *eba175,* for sequence amplification from parasite extracts. We identified a region within *eba175* as an ideal diagnostic target with a detection limit using standard gel-based PCR of 0.5 parasites per 1 x 10^{10} RBCs, followed by *msp2,* with a detection limit of one parasite per 1 x 10^{10} RBCs. All other targets exhibited a maximum detection limit of 20-30 parasites per 1 x 10^{10} RBCs. In order to cryopreserve late blood stage parasites and to improve the viability of early-stage parasites, we developed and analyzed several cryoprotectant formulations. We identified that glycerolyte,sorbitol and RPMI-based cryoprotectant preserved the viability of both early and mid blood stage parasites several-fold after a freeze-thaw cycle. These methods will be offered as improved standardized protocols for simplified parasite typing and cryopreservation.

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An orthologue of a Plasmodium vivax PHIST family member locates to the caveola-vesicle complexes in P. cynomolgi-infected erythrocytes

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In each Plasmodium species, the intracellular parasite forms networks within the red blood cell (RBC) cytoplasm, allowing communication between the parasite plasma membrane and the intra- and extra-erythrocytic environments enabling processes like nutrient import, protein export and RBC adherence. While knobs and Maurer’s clefts are observed in P. falciparum-infected erythrocytes, RBCs infected with other species Plasmodium display alterations such as caveolae, caveola-vesicle complexes (CVCs) and extensive cytoplasmic cleft membranes. Information on the antigenic makeup and function of these ultrastructural features is scarce. Building upon earlier published data showing that four P. vivax monoclonal antibodies (mAbs) recognized a 95 kDa antigen by SDS-PAGE and localized specifically to CVC membrane structures and cytoplasmic vesicles by immunoelectron microscopy, we

aimed to identify the genes encoding the corresponding proteins. Immunoprecipitation followed by trypsin peptide cleavage and LC-MS/MS analysis identified this protein as an 80.73 kDa member of the Plasmodium helical interspersed sub-telomeric (PHIST) superfamily of proteins. In addition to P. vivax and P. cynomolgi, the encoded protein has orthologues in P. knowlesi, and possible paralogues in P. falciparum, P. berghei and P. yoelii. All homologs contain a PEXEL motif in their amino acid sequence, conserved tryptophans, and four consecutive alpha helices. Immunoelectron tomography studies on P. cynomolgi-infected RBCs using rabbit antisera to the P. vivax CVC - PHIST protein revealed that the protein localizes to tubular extensions of the CVCs. Comparative studies of the CVC - PHIST and other proteins discovered by similar methodologies will advance the goal of understanding the functions of these structures, and the relevance of the observed morphological features shared amongst some Plasmodium species.

3104

A review of malaria-related deaths in US travelers, 2002-2008

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Malaria accounts for 1 million deaths per year worldwide and about half of the world's population live in areas with malaria. Travelers to these areas are also at risk but malaria is preventable. Malaria is also curable but depends on timely presentation, diagnosis, and treatment. Despite having state of the art medical care in the US, the annual number of malaria cases and case fatality rate (CFR) among US residents has plateaued over the past few years. In the US from 2002-2008, between 1250-1500 cases were reported, with about 60% occurring among US residents. The CFR among US residents had decreased from 0.9% in 2002 to 0.4% in 2004, but has since remained unchanged. We reviewed malaria-related deaths among US residents reported from 2002-2008 to examine potential contributing factors to malaria mortality in this population. Factors examined included delays in: seeking care (>2 days after symptom onset); diagnosis (>1 day between presentation and diagnosis); and treatment (>1 day between diagnosis and treatment). Information was incomplete for some patients. Of 34 deaths reported, 24 were US residents. Patients were mostly male (n=17, 70%) with a median age of 24 (range 19-69 years). *P. falciparum* (n=18, 75%) caused most cases. Top reasons for traveling were missionary work (n=8, 30%) and to visit friends and relatives (n=7, 29%). Most patients (n=15/21, 68%) used no prophylaxis. Of the 6 on prophylaxis, only 2 were on an appropriate drug, but neither adhered. Delays in seeking care and diagnosis occurred in 73% (n=16/22) and 43% (n=9/21) of patients, respectively. There were no treatment delays but 56% (n=10/18) received an inappropriate drug (quinidine unavailable for 4). Preventable factors were present in malaria deaths among US residents from 2002-2008. Education is needed for both US travelers and physicians on malaria prevention, and for physicians on malaria management. Furthermore, the instances of quinidine unavailability highlight the need for hospitals to keep quinidine on formulary and for an alternate intravenous anti-malarial in the US.

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***Taenia solium* infections of humans and pigs in Tanzania and Mozambique**

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Community-based epidemiological surveys were conducted on *Taenia solium* infections of pigs and humans in Mbeya Region in the southern highlands of Tanzania and Tete Province in northwestern Mozambique under the auspices of the "Cross-Disciplinary Risk Assessment of *Taenia solium* Cysticercosis in Eastern and Southern Africa (CESA)" project. Lingual examination of pigs was conducted and serum samples collected for testing by antigen detecting ELISA (Ag-ELISA) for porcine cysticercosis. In Tanzania the overall prevalence of porcine cysticercosis in Mbozi district was 11.7% and 32% based on lingual examination and Ag-ELISA testing, respectively, of 300 pigs while in Mbeya Rural district, the prevalences were 6% and 30.7% by lingual examination and Ag-ELISA testing, respectively, of 300 pigs. In Mozambique the prevalence of porcine cysticercosis was 12.7% and 34.9% based on lingual examination and Ag-ELISA testing, respectively, of 661 pigs in Angonia District. A serological survey of 1723 persons from the same communities in Angonia indicated 14.5% had active cysticercosis by Ag-ELISA. Computerised tomography (CT) scanning of Ag-ELISA positive epileptics indicated that 70.6% had neurocysticercosis (NCC) while only 17.8% of Ag-ELISA negative epileptics were found to have NCC. In Tanzania a serological survey of 830 persons in Mbozi District communities indicated 45.3% positive for exposure to cysticercosis by the CDC's rT-24h antibody detecting ELISA while 16.5% were found positive for active cysticercosis infection by Ag-ELISA. Copro-antigen testing of fecal samples collected from 820 of these same persons indicated 5.2% had active *Taenia* tapeworm infection. Our data resulting from these community-based surveys in rural pig producing areas of Tanzania and Mozambique indicate that *T. solium* cysticercosis is indeed emerging as a serious public health and agricultural problem in eastern and southern Africa as evidenced by these high prevalences of both human and porcine cysticercosis. Urgent attention is needed to further understanding of the cysticercosis situation in the region and mobilize efforts to combat the disease.

3106

Dengue Virus Seroprevalence Among Febrile Patients in Bamako, Mali: Results of a 2006 Surveillance Study.

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The dengue viruses (DENVs) constitute a significant public health threat, annually infecting 50 to 100 million individuals worldwide. However, limited surveillance information exists for many potentially high-risk areas including the subtropical West African country of Mali. In order to assess the need for ongoing DENV surveillance in the region, 96 human serum samples were collected from the serum bank at the National Research Institute in Public Health (INRSP) in Bamako, Mali. DENV-specific IgM and IgG antibody capture ELISAs were performed on all samples, and a subset of IgG positive samples were tested using the plaque-reduction neutralization test (PRNT). Samples collected during acute infection (0-5 days post onset of symptoms) were tested using the Platelia Dengue NS1 Antigen Capture ELISA (Bio-Rad Laboratories, Marnes-La-Coquette, France). Additionally, acute samples were inoculated into C6/36 cells and cell supernatant was analyzed using reverse-transcriptase polymerase chain reaction (RT-PCR) for flaviviruses, alphaviruses, and bunyaviruses. Although we found no DENV IgM positive samples, 93.5% (87/93) of samples tested were positive for DENV IgG. While no PCR positives were found, we were able to detect DENV NS1 using the Bio-Rad kit in 1 of the 20 acute samples tested, indicating an acute DENV infection. These results, coupled with the October-November 2008 DENV outbreak in the Kayes region of western Mali, emphasize the need for continued DENV surveillance in Mali.

3107

Nimodipine markedly increases efficacy of artemether in rescue treatment of cerebral malaria in Plasmodium berghei ANKA-infected mice

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Cerebral malaria (CM) is a major complication of Plasmodium falciparum infections, responsible for an estimated one million deaths every year mainly of children under 5 years old. In addition, survivor children may face permanent neurological and cognitive deficits. Adjunctive therapies that improve the rate and the quality of survival of CM patients upon antimalarial treatment are scarce, and the development of new treatment strategies based on the knowledge of the pathogenesis process is therefore urgently needed. The brain microcirculation is a major target in CM pathogenesis and in this work we used the murine model of CM by Plasmodium berghei ANKA to study the dynamic cerebral microcirculatory changes occurring during infection, by directly assessing the pial microvasculature by intravital microscopy through a closed cranial window. We show that murine CM is associated with marked decreases (mean: 60%) of pial arteriolar blood flow due to vasoconstriction and decreased blood velocity. Leukocyte sequestration further decreased perfusion by narrowing luminal diameters in the affected vessels and blocking capillaries. Remarkably, vascular collapse at various degrees was observed in 44% of mice with CM which also presented more severe vasoconstriction. Co-administration of artemether and nimodipine, a calcium channel blocker used to treat post-subarachnoid hemorrhage vasospasm, to mice presenting CM markedly increased survival (60-66%) compared to artemether plus vehicle only (32%). Administration of nimodipine induced vasodilation and increased pial blood flow. We conclude that vasoconstriction and vascular collapse play a role in murine CM pathogenesis and nimodipine holds potential as adjunctive therapy for CM.

3108

Application of real-time quantitative PCR (qPCR) for endpoint determination in anti-malarial drug trial

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The determination of study endpoints in clinical trials of anti-malarial interventions have traditionally relied on slide microscopy. However, blood film microscopy is slow, has low sensitivity and is subject to operator-induced variability which may compromise accurate comparison of different intervention arms. PCR-based methods which have much higher sensitivity and are less sensitive to operator bias as well as amenable to high throughput application would greatly facilitate clinical trials of anti-malarial interventions. We compared real-time quantitative PCR (qPCR) assay with microscopy for malaria diagnosis and estimation of parasite density in 1211 hospital patients and subsequently evaluated the performance of both methods for determination of parasite clearance time (PCT) in an efficacy trial of two anti-malarial drugs. Blood samples collected from 106 study patients at 8-hourly intervals over a 3-day period (~1060 blood samples) were analyzed by qPCR amplification of *Plasmodium falciparum* 18S rDNA and microscopic

examination of giemsa-stained blood films.
Agreement between microscopic and qPCR diagnosis (Kappa=0.86; 95% CI = 0.83-0.90) as well as concordance of parasite density estimates (rho_c=0.97; 95% CI= 0.96-0.97) were very high. However, estimates of parasite clearance time differed between the two methods with microscopy indicating a median PCT of 16H compared to 24H by qPCR. All patients appeared to have cleared their infection by day 3 post-treatment judging by microscopy, although 19% (16/83) still harboured asexual parasitaemia detectable by qPCR.
These results suggest that applying a sensitive parasite detection method could lead to more precise determination of the relative efficacies of different anti-malarial interventions.

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Over-expression of *Rel2* in transgenic mosquitoes and *Rel2* mediated *Plasmodium* resistance

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Mosquitoes transmit a broad range of human parasitic and viral diseases, within which malaria is the most devastating insect-borne disease. A major bottleneck for *Plasmodium's* development occurs during the ookinete invasion of the midgut epithelium, prior to the development of oocysts on the basal lamina. The mosquito's innate immune system has been shown to play an important role in killing parasites at this stage of infection and IMD pathway plays an essential role in mounting the anti-*Plasmodium* immune responses. Our previous results showed that gene silencing of the negative regulator of *Rel2*, *Caspar*, which allows the transient stimulation of the IMD pathway, resulted in almost complete refractoriness of three major malaria vectors, *Anopheles gambiae*, *A. stephensi,* and *A. albimanus* mosquitoes to the human malaria parasite *Plasmodium falciparum*. In this study, by using piggyBac based vector we generated 12 independent transgenic lines of *A. stephensi* over-expressing the transcription factor *Rel2* which was originated from *A. gambiae* mosquitoes. The transgene is under the midgut-specific blood meal inducible carboxypeptidase promoter. PCR and southern hybridization have confirmed the insertion event of the transgene in the individual lines. Importantly, induction of transgenic Rel2 leads to the significant decrease in susceptibility of *A. stephensi* to *P. falciparum* infection. All 12 lines of transgenic mosquitoes have shown significant resistance to malaria parasites with different level of intensities, line #2 and #15 have shown the strongest resistance to the *P. falciparum* infection, while line #1 has shown the least resistance to *P. falciparum* infection. The expression profile of the effector genes upon the induction of Rel2 in the transgenic mosquitoes, and the resistance of transgenic mosquitoes to both Gram negative and Gram positive bacterial infection will be assessed. For the long term goal, we aim at using these transgenic mosquitoes to dissect the molecular mechanism of Rel2-mediated anti-*Plasmodium* activity in the anopheline mosquitoes.

3110

Transcriptomic Analysis of Host Response to Giardia lamblia Infection Reveals a Role for Mannose Binding Lectin in Parasite Control

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Infection with *Giardia lamblia* is one of the most common causes of diarrheal disease in the world. While numerous studies have identified important contributions of adaptive immune responses to parasite control, much less work has examined innate immunity and its connections to the adaptive response during this infection. To gain a global perspective on responses to *Giardia* infection, we applied microarray technology to profile intestinal gene expression in C57BL/6 mice following infection with *G. lamblia* trophozoites (strain GS (M)-H7). A total of 96 transcripts were identified as being significantly regulated. Induced transcripts were categorized as coming primarily from B cells and mast cells, consistent with prior knowledge of this infection. Other regulated transcripts suggested activation of Paneth cells and innate immunity. We further explored the role of one induced transcript, mannose-binding lectin (Mbl2). Wild type mice expressed higher levels of Mbl2 upon infection compared with uninfected mice. In contrast, TNF-deficient mice, which have a defect in the clearance of *Giardia*, did not induce Mbl2 transcripts. Mbl-deficient mice showed a reduced ability to recruit mast cells in the intestinal submucosa and were delayed in their elimination of *Giardia* infection. These data have identified a new mechanism contributing to control of *Giardia* infection, recruitment of mast cells to the intestinal tract by Mbl, and suggest a potential mechanism contributing to the variation in clinical symptoms associated with giardiasis.

3111

The Economic Benefits Resulting from the First 8 Years of the Global Programme To Eliminate Lymphatic Filariasis (2000-2007)

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The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was initiated by WHO in 2000 to stop the spread of filarial parasite infections that put 1.3 billion people in over 80 countries at risk of acquiring lymphedema, elephantiasis, hydrocele and other

manifestations of LF. Following a strategy of once-yearly, single-dose, 2-drug administration (albendazole + either ivermectin or diethylcarbamazine) to all at-risk for 4-6 years, the GPELF delivered more than 1.9 billion treatments to almost 600 million at-risk individuals in its first 8 years. To calculate the resulting economic benefits of these achievements, the number of clinical manifestations averted was quantified and the savings associated with this disease prevention analyzed in the context of direct treatment costs, indirect costs of lost-labor, and costs to the health system to care for affected individuals.
This study estimates that US$21.8 billion of economic benefits will be gained over the lifetime of 31.4 million individuals treated during the first 8 years of the GPELF. Over US$2.3 billion is realized by nearly 3 million newborns and other individuals protected from acquiring LF because of being born in areas freed of LF transmission. Similarly, more than 28 million individuals already infected with LF benefit from MDA halting the progression of their disease, resulting in an associated economic benefit of approximately US$19.5 billion. In addition to these economic benefits to at-risk individuals, reduced patient services associated with LF morbidity save the health systems of endemic countries approximately US$2.2 billion.
The true economic value of the GPELF is even greater after factoring in the difficult-to-quantify quality of life improvements and prevention of other LF syndromes and co-endemic diseases. Given that program implementation costs have been previously determined to be very low, it is clear that the economic rate of return of the GPELF is extremely high and ensures that this Programme will continue to prove itself as an excellent investment in global health.

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Immunogenicity of a Blood Stage Malaria Vaccine Candidate, BSAM-2, Formulated in GLA-SE with and without Alhydrogel

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AMA1 and MSP1(42) are considered leading asexual blood-stage vaccine candidates. Multiple human trials indicated safety and moderate immunogenicity when recombinant AMA1 and MSP1(42) were formulated separately. The Blood Stage Antigen Mixture-2 (BSAM-2), composed of recombinant AMA1-FVO, AMA1-3D7, MSP1(42)-FVO and MSP1(42)-3D7 in 1:1:1:1 mass ratio, may provide better immune coverage resulting in a vaccine with greater efficacy than either antigen alone. BSAM-2 formulated on Alhydrogel in combination with the Toll-like receptor (TLR) 9 ligand CPG 7909 is currently being tested in a Phase 1 clinical trial. The goal of the current study was to evaluate the possibility of enhancing the immune response to BSAM-2 using an alternative TLR ligand. BSAM-2 was formulated with EM005, a synthetic TLR4 agonist in a stable oil-in-water emulsion. BSAM-2 was also adsorbed to Alhydrogel followed by mixing with EM005. The latter formulation was designed to stabilize the antigens in the EM005 formulation. CD1 mice were immunized with these BSAM-2 formulations with several antigen and adjuvant dose levels. Antibody responses were analyzed by ELISA. Significantly higher antibody levels were induced to both AMA1 and MSP1 in groups that did not contain Alhydrogel, suggesting an inhibitory effect when antigens were adsorbed to Alhydrogel prior to formulation with EM005. Isotyping analyses indicate that IgG3 levels produced in EM005 groups are reduced when Alhydrogel is included in the formulation. Intracellular cytokine staining assay also indicated a lower T-cell response in the Alhydrogel groups. This data indicates Alhydrogel had a negative impact on the immunogenicity of the BSAM-2/EM005 formulation. Studies are ongoing in other animal models to elucidate these findings.

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Relationship of Lassa Fever Knowledge and *Mastomys* Rodent Abundance in Kenema District, Sierra Leone

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The arenavirus Lassa (LASV) causes Lassa fever (LF), an acute hemorrhagic illness endemic to West Africa. Primary transmission of LASV occurs through contact with blood or excreta of the rodent reservoir, *Mastomys natalensis*, which is found in close association with human populations. Secondary human-to-human transmission also occurs. We conducted a survey on knowledge of LF and assessed rodent abundance to guide measures for LF control in a hyperendemic area for the disease in eastern Sierra Leone. Six villages with confirmed cases of LF in the previous 6 months were chosen for study. A questionnaire was administered to the first available adult in each household and one Sherman trap was placed in each bedroom for two nights. Questionnaires were administered and traps set in a total of 355 and 318 houses, respectively. Eighty nine percent of respondents had heard of LF and 79% knew rodents transmit LASV. Respondents who knew that rodents transmit LASV were more likely to have reported implementing rodent control measures (OR: 2.6, 95%CI: 1.3-5.2). In 1,891 trap nights, 260 animals were collected, of which 177 were *Mastomys* (overall and *Mastomys*-specific trap successes 13.7% and 9.4%, respectively). Species identification by PCR is pending. Overall and *Mastomys*-specific abundance were lower in houses reporting implementation of rodent control measures (p=0.003). In contrast to previous studies which associated *Mastomys* abundance and LF risk to crowding, we found no correlation between household population density and rodent abundance when we controlled for size of house (r=0.101, p=0.07). Our results indicate that reduction of *Mastomys* is possible in this resource-poor setting through community-initiated control. The impact of these control measures on the incidence of LF has yet to be determined. Further studies are needed to evaluate effective, sustainable, and culturally acceptable methods to reduce *Mastomys* abundance and LASV transmission.

3114

Activity and precision of excision the *Mos-1 mariner* transposon in *Schistosoma mansoni*

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The genome sequence of the schistosome *Schistosoma mansoni* is now available in draft format. The genome includes at least 11,800 protein encoding genes. Many of these genes represent new targets for intervention for treatment and control of schistosomiasis. There is a role for transgenesis technologies in characterizing the function of schistosome genes. In forward genetics approaches, exogenous transposons have been shown to be powerful transgenesis tools in other species. We have demonstrated previously that the *piggyBac* transposon is active in schistosome tissues and can integrate into the schistosome genome (Morales et al. 2007 *FASEB J*. 21, 3479-3489).
The *Mos-1 mariner* transposon, originally isolated from the fruit fly has been widely used for transgenesis and for genetics. Given its activity in diverse species, we are investigating the activity of *Mos-1 mariner* in schistosomes and comparing its performance with *piggyBac*. Here we examined the precision of excision of *Mos 1* from the donor plasmid backbones after introduction of the transposon by square wave electroporation into schistosomules along with mRNA encoding the cognate transposase. We observed that *Mos 1* cassette was excised from the plasmid backbone, demonstrating for the first time that *Mos 1* was transpositionally active in schistosome tissues. Excision of *Mos1* was not precise. Only one of 15 excised plasmids displayed precise excision from within a TA dinucleotide; six of the15 included parts of the transposon's inverted terminal repeat (ITR) and eight others revealed excision of plasmid backbone sequences exterior and adjacent to the ITR. Furthermore, contrary to the precision of excision of *piggyBac* reported in several other target species, excision of *piggyBac* - like with *Mos 1* - also was imprecise. Donor cleavage of the *piggyBac* constructs occurred at a different sequences from the standard TTAA site. In particular, 0 of 13 excised plasmids displayed precise excision from within a TTAA motif; seven included part of the *piggyBac* ITR, ranging in length from 8 - 62 bp and six others showing excision of plasmid backbone sequences near the ITR.
Nonetheless, imprecise excision (and/or imprecise chromosomal insertion) may not disrupt the reporter transgene within the donor cassette. We now are investigating reporter gene activity and integration of *Mos1* into the chromosomes of schistosomes, and its potential for vertical transmission to schistosome progeny.

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Dengue risk along an altitudinal gradient in Venezuela

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The incidence of dengue fever and dengue hemorrhagic fever has been increasing worldwide since the 1950s, and has expanded throughout Latin America since its emergence there in the 1980s. In 2007, there were over 890,000 cases of dengue fever in the Americas, with greater than 80,000 cases occurring in Venezuela. In recent decades one of the major factors driving the recolonization of *A. aegypti* and the reemergence of dengue fever in the Americas is rapid and uncontrolled urbanization. Attempts to maintain housing standards, potable water access, and trash collection have not kept pace with the explosive growth of Latin American cities. All three of these deficiencies are correlated with *A. aegypti* larval densities, as rainwater accumulated in trash and debris, constitutes ideal larval habitat. While urbanization results in increased abundance of *A. aegypti* breeding habitat, climate may limit their distribution in the Andean sub-region of Venezuela. We studied *A. aegypti* distribution in three cities along an altitudinal gradient in the Venezuelan Andes, Merida state. We evaluated the relative influence of altitude (and by proxy, climate) and neighborhood level variables such as human demographics, accessibility of potable water, regularity of trash collection, vegetation cover, and recent history of land utilization on the prevalence of *A. aegypti*. We randomly selected a block in each of 24 neighborhoods, where we surveyed all houses for larvae and conducted socioeconomic surveys during the summer of 2008. We found a significant, negative effect of altitude on the container index (number of positive containers per 100 containers), even after controlling for other variables. The highest positive container was found at 1900m; exhaustive searches in two cemeteries at higher altitudes yielded no larvae. Out study indicates that *A. aegypti* is limited by climate at these higher altitudes. This information may provide insights regarding the effects of climate change on the spread of dengue virus and other infectious diseases.

3116

Development and Optimization of Novel Anti-Flavivirus Compounds

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Flaviviruses (family *Flaviviridae*, genus *Flavivirus*) are a significant cause of morbidity and mortality world-wide and are considered potential bioweapons due to ease of transmission via mosquito vectors and a lack of effective therapeutic options. Despite the high human and economic costs of flavivirus infections there are currently no safe and cost-effective therapeutic agents available to treat

these diseases. We are targeting the NS5 capping enzyme (CE) of Dengue (DEN) and yellow fever (YF) viruses in order to develop an antiviral treatment that will inhibit the deadly diseases caused by these viruses. The CE is responsible for the formation of the 5' cap to the viral (v) RNA. The 5' cap structure functions to protect the vRNA from degradation by 5' exoribonucleases and to direct translation of the viral polyprotein. Both of these functions are essential for virus replication, and as such the CE is a valuable target for antiviral drug discovery. The identification of a small molecule that could compete for the binding site on the CE would prevent viral replication and allow for the virus to be cleared from the body without causing severe disease. We have performed a high-throughput screen at the National Screening Laboratory (NSRB) of ~280,000 compounds and identified ~350 compounds that show potential GTP displacement activity. We are in the process of further testing these compounds to determine biochemical activity and in replicon assays to assess antiviral activity. In addition, each of the compounds is being computationally docked into the structure of the capping enzyme GTP-binding site and analyzed *in silico* to determine probable modes of association and to suggest new analog compounds predicted to possess improved binding and drug-like characteristics. We are currently developing structure-activity relationship data for compounds that display biochemical and antiviral activity in a highly iterative process, and the results of these studies should result in the identification of a novel class of antiviral compounds useful for the treatment of flaviviral infection.

3117

Factors affecting drinking water choices in rural Ghana after the introduction of Small Water Enterprises

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The Johns Hopkins University Center for Water and Health is evaluating Small Water Enterprises (SWEs) in five Ghanaian villages. SWEs are found throughout the world vending water to households (HH) in regions beyond the reach of piped water systems. Their ubiquity in the developing world suggests that they may prove valuable in improving potable water availability and access to vulnerable populations. The community-based SWEs currently under study utilize advanced water kiosks that house sand filtration, activated carbon and ultraviolet light to treat surface water. Each village-level SWE aims to provide access to drinking water that meets World Health Organization (WHO) microbial guidelines at competitive market prices to HHs living below the poverty line.
In each of five villages the study consisted of a randomized selection of 100 GIS-coded HHs, key informant interviews and microbial and chemical testing of kiosk water as well as alternative drinking water sources. HH surveys (n = 501) of female caretakers within study villages found that water vended by the SWE, meeting WHO microbial guidelines and sold at a relatively low-cost (approximately USD 0.05 per 18 liter container) was often bypassed for water that was of lower quality and/or more expensive. Household drinking water choices were found to be made based on multiple factors including taste, distance between household and water source as determined by GPS tracking, perceived water quality, cost, belief systems and ancestral behaviors. In many instances, treated kiosk water was purchased for household washing rather than for drinking. These findings reinforce the need for community mobilization and behavior change communication programs to be embedded within SWE drinking water interventions to impact water collection behaviors. Populations must be aware of the importance of drinking microbiologically safe water to prevent disease and reduce childhood morbidity and mortality.

3118

Full recovery after initial delay of IV Artesunate treatment in a 14-year old boy with 12 of 14 manifestations of Severe *P. falciparum* Malaria

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A previously healthy 14 year old boy was admitted with 5 days of worsening headache, fever, chills, fatigue, emesis and 1 day of progressive somnolence. He arrived from Nigeria 22 days before admission, after a 2 year stay. He was empirically prescribed Mefloquine at a different facility 5 days prior, but the only outpatient dose of 250mg was taken on day of admission.
At admission, patient was febrile (39^{o}C), tachycardic, hypotensive, and had a GCS of 10. Thick and thin malaria smears showed 18% ring parasitemia including multiple trophozoites per erythrocyte and occasional banana-shaped gametocytes. Immediate citywide search for IV Quinidine revealed none, so CDC was contacted for IVArtesunate, which arrived by air transport 12 hrs later. Within 3 hrs of admission patient met 12 of 14 WHO signs/symptoms of severe malaria: unrousable coma cerebral malaria, hyperparasitemia, high fever, severe anemia, metabolic acidosis, disseminated intravascular coagulation, fluid and electrolyte disturbances, pulmonary edema, circulatory collapse, jaundice, renal failure and macroscopic hemoglobinuria (“Blackwater fever”). While waiting for Artesunate, patient was intubated in the ICU, and three medications with some anti-malarial effect were given: Mefloquine 1gr. via NGT, Doxycycline 100mg IV q12hrs, and Ciprofloxacin 500mg IV. Exchange transfusion was considered but IV Artesunate arrived first and was given in four doses of 2.4mg/kg/dose IV bolus at times 0hrs, 12hrs, 24hrs and 48hrs. Six hrs after its the first Artesunate dose, parasitemia decreased from 18% to <2% and by 24hrs it was <1%. After 30hrs the patient was extubated, by 48hrs had a GCS of 14 and his urine cleared. At discharge on day 7, he had complete recovery.
This adolescent boy clinically behaved more as an adult than as a child with severe malaria. Clinical adult profile included longer duration of symptoms before features of severe disease developed (renal failure, jaundice, pulmonary edema). One feature closer to a pediatric clinical response was the fast recovery of 1-2 days from initiation of treatment to 2-3 days seen in adults.

3119

Screening for Chagas Antibodies using Blood Collected and Stored on Filter Paper

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As part of a small scale serological survey near an autochthonous case of *Trypanosoma cruzi* in southern Louisiana (see late breaker by Lopez, et al), we modified the Chagatest ELISA recombinant V 3.0 (Weiner Lab, Rosario, Argentina) for use with blood collected on filter paper (Whatman 903 Protein Saver Cards, Whatman Ltd, Kent, UK). Due to difficulty in obtaining quantities of whole blood in population-based studies, we evaluated the collection of blood samples on filter paper to be used for lab-based confirmatory testing. Following collection, filter papers were stored at -20°C until testing; samples were then eluted from the filter paper and tested by the Chagatest ELISA recombinant V 3.0. This method proved to be equivalent in accuracy to testing serum samples in the ELISA test procedure and shows promise as a new technique for Chagas antibody testing allowing easy and efficient sample collection and storage.

3120

Nest-Box Mosquito Trap: evaluation of a novel ornithophagic mosquito collection device

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Recently a high infection prevalence of *West Nile virus* (WNV) was demonstrated among breeding populations of Prothonotary warblers (*Protonotaria citrea*) in Central Virginia. In an attempt to understand the elevated WNV infection prevalence, we designed a novel Nest Box Mosquito Trap (NBMT) to evaluate the mosquito biting rate on nestling and nest-attending adult Prothonotary warblers. This study evaluated the collection efficacy of NBMT under controlled laboratory conditions using lab-reared *Aedes aegypti*. In the field, NBMT collected 19 warbler-seeking *Culex salinarius* mosquitoes as opposed to 1 host-seeking *Cx. erraticus* collected in the control (unoccupied) boxes over a series of 30 trap nights per group. Finally, to determine the total time the warblers were present to attract host-seeking mosquitoes and to ensure the device did not disrupt bird nesting behavior we recorded warbler nest temperature at minute intervals (Thermochron i-Buttons, Embedded Data Systems). The Nest Box Mosquito Trap does not appear to significantly alter Prothonotary warbler nest attendance behavior and is an effective tool in determining the mosquito biting rate on nesting avian fauna. Future studies evaluating the mosquito biting rates of other nesting avian species are planned.

3121

A QTN in the LaCrosse virus NSm gene is associated with stabilized super-infection of *Aedes triseriatus* mosquitoes.

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LaCrosse virus (LACV) infection rates in mosquitoes collected as eggs or larvae from the field seem insufficient to maintain the virus in nature. To investigate this issue, we reared mosquitoes from field-collected eggs and assayed adults individually for LACV antigen by immunofluorescence, viral nucleic acid by qPCR, and infectious virus. The mosquitoes segregated into three infection phenotypes: 1) super-infected (SI+) mosquitoes that contained infectious virus, large accumulations of viral antigen and viral nucleic acid and comprised 0.08% of the mosquitoes, 2) infected mosquitoes (I+) that contained no detectable infectious virus in cell culture assays, lesser amounts of viral antigen and nucleic acid, and comprised approximately 3.8% of the mosquitoes, and 3) non-infected mosquitoes (I-) that contained no detectable viral antigen, nucleic acid, or infectious virus and comprised 96.1% of the assayed mosquitoes. The SI+ mosquitoes may represent lineages of stably-infected *Ae. triseriatus* in nature. Sequence analyses of genomes of LACV isolates and amplified viral RNA sequences from SI+ and I+ mosquitoes, respectively, suggested that the NSm (a potential suppressor of apoptosis), but not the NSs gene (a potential suppressor of RNAi *in vivo*) may condition the respective phenotypes. The NSm gene is highly polymorphic, and a QTN at Position 247 corresponds to a U çè A transversion in the third position of a CUN Leu codon.
This codon shows severe bias towards CUU (57 codons in 14 mosquitoes) compared to CUA (7 codons in 14 mosquitoes). All 7 CUA codons appeared in superinfected mosquitoes, none appeared in the infected mosquitoes (p-value = 0.01437). In contrast the NSs :LACV gene is absolutely conserved among SI+ and I+ mosquitoes. These results provide possible innate immune correlates of I+ vs. SI+ phenotype. The results are suggestive of the gene for gene innate immune arms race between Sigma virus and the *ref(2)P* innate immune gene of *D. melanogaster*, which conditions stabilized infection of the arthropod host and maintenance of the virus in nature without amplification in vertebrate hosts.

3122

Determination of viability and parasite burden of *Leishmania* in clinical samples using RT-qPCR by 7SLRNA

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Background Molecular detection of *Leishmania* DNA is highly sensitive and specific. Amplification of *L. Viannia* kDNA from blood, normal skin and mucosa of patients with cutaneous leishmaiasis documented wide dissemination of parasites during active disease and persistence after clinical resolution However, the clinical, biological and epidemiological significance of the presence of parasite DNA is controversial. Short half-life and lability of RNA makes it a plausible marker of viability. We exploited 7SLRNA, an RNA/protein complex involved in intracellular protein translocation to establish viability and quantify parasites.
Methods Proof of concept was achieved by real-time PCR of *L. Viannia* 7SLRNA and parallel evaluation of luciferase activity in *luc* transfected intracellular amastigotes in dose-response assays of Glucantime® cytotoxicity. Clinical samples (monocytes, aspirates of normal skin and lesions, tonsil swabs) from 30 patients with cutaneous leishmaniasis were examined by kDNA amplification with LVB1 primers/Southern blot analysis. Positive samples were analyzed by real time PCR to detect the 7SLRNA gene (qPCR) and transcripts (RT-qPCR).
Results Number of 7SLRNA copies and antimony concentration were inversely related. Dose-response curves of 7SLRNA amplification and luminometry coincided. The 7SLRNA gene and its transcripts were detected in a high proportion of kDNA positive samples. 7SLRNA indicating living parasites was detected in 68% (13/19) of blood samples, 42% (5/12) of tonsil swabs and in 36% (4/11) of healthy skin aspirates and 92% (23/25) lesion aspirates. Quantification of 7SLRNA gene copies evidencing parasite load was achieved in 92% (12/13) of tonsil swabs, 79% (15/19) of monocyte samples and 73% (8/11) of healthy skin aspirates. Parasite load in non lesion samples varied from 7.000 - 35 million being highest in blood monocytes.
During active cutaneous leishmaniasis, viable *Leishmania* are demonstrable in unaffected tissues including blood, mucosa and skin. 7SLRNA and its gene are an informative targets for clinical and epidemiologic studies.

3123

Transmission and longevity of the *piggyBac* transposon in transformed *Schistosoma mansoni*

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We have demonstrated previously that the *piggyBac (PB)* transposon is active in schistosome tissues and can integrate into the genome. Using this binary *PB* transposon system, we are now investigating transgene delivery, transmission and longevity of the *PB* transposon in developmental stages of *S. mansoni*. In one approach, we targeted the asexual phase of the developmental cycle in the intermediate host snail, with the aim of establishment of vertical transmission of *PB* transgenes from egg to cercaria. The eggs were transformed with either circular or linearized *PB* donor plasmid (containing a luciferase reporter) together with mRNA encoding the *PB* transposase by electroporation. Miracidia hatched two days later from these eggs were permitted to infect *B. glabrata* snails by the natural route. This process was repeated four times, A, B, C, and D. Each group included 20 to 30 snails that were exposed to >10 transformed miracidia per snail. Five to 10 weeks later, cercariae were shed from the infected snails and genomic DNAs was extracted from them. PCR targeting the luciferase transgene was employed to investigate the presence of transgenic cercariae by analysis of the gDNAs. From two of the four groups, A and D, snails shed cercariae positive for the luciferase transgene. Given the complexity of schistosome development in the snail, through mother sporocyst, production of daughter sporocysts which in turn produce cercariae, and the similar findings observed on two separate occasions, the presence of the luciferase transgene in these gDNAs suggests that the transposon may have been transmitted vertically from transformed eggs to cercariae via integration of the germ line genome.
In a second approach, we are targeting the sexual phase of the developmental cycle in the definitive host mouse, with the aim of establishment of germline transmission of *PB* transgenes from schistosomule through meiosis in the adults to the egg. After transformation of schistosomules with PB (as above), mice were infected by peritoneal inoculation. Ten weeks later, adult worms and eggs were recovered from the infected mice. Southern hybridization demonstrated the presence of the luciferase transgenes in gDNA from the mixed sex adult worms, indicating survival of the transgene for at least 10 weeks. For both approaches, we are analyzing these gDNAs by Southern hybridization, anchored PCR, and quantitative PCR approaches for evidence of *PB* transgene integrations.

3124

HbE confers protection against life-threatening Plasmodium falciparum malaria in Cambodia by impairing the cytoadherence of parasitized red blood cells

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Hemoglobin (Hb) E differs from normal HbA by a glutamate-to-lysine substitution at position 26 in the beta-globin chain. The high prevalence of HbE in malarious areas of Southeast Asia suggests that HbE protects against severe P. falciparum malaria. To test this hypothesis, we conducted a case-control study in Cambodia where 40% of individuals carries HbE. We compared HbE prevalence between severe malaria 'cases' and uncomplicated malaria 'controls'. We estimated the odds ratio (OR) of severe malaria in HbE compared to HbA individuals by logistic regression analyses that accounted for alpha-thalassemia and three correlates of immunity: age, male sex, and residence near forested areas where P. falciparum is transmitted. We additionally tested for an effect of HbE on 12 other malaria-related syndromes or groups of syndromes. Results from 750 patients suggest that HbE reduces the odds of cerebral malaria by 50% (OR 0.53, 95% CI 0.27-0.96, P=0.047, uncorrected for multiple testing). HbE protection against severe malaria was not associated with reduced parasite density, suggesting that HbE does not strongly restrict parasite multiplication in vivo. Alternatively, we hypothesized that HbE impairs the adherence of parasitized red blood cells (RBC) to microvascular endothelial cells (MVEC) - a host-pathogen interaction critical to the development of severe disease. To test this possibility, we obtained ring-infected RBC and cultured them to trophozoite-infected RBC. We then purified these parasitized RBC and used them to infect AA, AE, and EE RBC. After parasite invasion and development to trophozoites expressing PfEMP1 (the parasite's main cytoadherence ligand), we compared the adherence of parasitized RBC to MVEC. Compared to parasitized AA RBC, parasitized AE and EE RBC showed 40% and 60% reduced binding to MVEC (P<0.0001). Reduced binding was associated with fewer 'knobs' in which PfEMP1 is concentrated on the surface of parasitized RBC. Our data suggest that HbE enables parasitized RBC to bind MVEC avidly enough to sequester from the spleen and multiply to high densities, but not avidly enough to induce high levels of microvascular inflammation associated with severe malaria.

3125

Post-translational modification of tau protein in the brains of mice infected with *P. berghei* ANKA

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Cerebral malaria (CM) results in an encephalopathy often associated with seizures, obtundation and alterations in mental status. Previously we demonstrated that infection of C57BL/6 mice with *Plasmodium berghei* ANKA (PbA) was associated with a vasculopathy with increased endothelin levels in the brain resulting in decreased cerebral blood flow and axonal damage. This resulted in measurable cognitive deficits in infected mice. However the mechanisms of neuronal damage have yet to be elucidated. Here, we present data demonstrating an increase in aberrant phosphorylation of tau protein in the brains of mice with CM. Tau is a microtubule-associated protein in the brain involved in axonal transport and neuronal function. When abnormally phosphorylated, these proteins undergo structural changes and accumulate in neuronal cell bodies forming the neurofibrillary tangles such as observed in Alzheimer's disease and other fronto-temporal dementias. In our model, the abnormal tau protein phosphorylation is associated with abnormal regulation of the AKT/GSK3β signaling pathway during acute infection with PbA. This is associated with a global decrease in glucose uptake in the brains of infected mice when compared with controls as measured by microPET imaging despite comparable serum glucose levels in control and infected mice. These data lend credence to the idea that the ischemia caused by the vasculopathy associated with CM leads to metabolic dysfunction in the brain involving the insulin signaling pathway and that this results in abnormal tau phosphorylation in the brains of infected mice. These observations may have implications for human CM.

3126

Synthetic peptide inhibition of dengue virus infection in
Aedes aegypti mosquitoes

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Dengue virus (DENV) is an arthropod-borne Flavivirus of global public health importance. It causes more human morbidity and mortality than any other Flavivirus resulting from an estimated 100 million infections, including some 500,000 cases of the more severe hemorrhagic manifestation of the disease. The need for a remedy is as significant as the global burden of this disease: there is currently no effective treatment and no commercially available vaccine. Moreover, as vaccine candidates must elicit immunity to all four viral serotypes, this added technical difficulty to the development process has made alternative therapies greatly desired. This study investigated the ability of a synthetic peptide (DN59) derived from the DENV E glycoprotein, previously shown to inhibit viral cell entry and infection in mammalian culture, to inhibit virus infection in Aedes aegypti mosquitoes, the natural virus vector. Mosquitoes were intrathoracically injected with either a combination of DN59 peptide and DENV, or with DENV alone. The subsequent degree of systemic viral infection was monitored with quantitative real time PCR (qRT-PCR) over 12 days to assess peptide inhibition properties over time. Preliminary results suggested DN59 significantly inhibited viral infection in Aedes aegypti (p=0.03). There was an approximate 50% reduction of detectable DENV RNA in DN59 treated groups. The potential for use of this peptide in disease prevention, and the biological significance of these results will be discussed.

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Spatial and temporal analysis of West Nile virus hot spots in South Dakota

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West Nile virus invaded the Northern Great Plains in 2002 and caused a significant outbreak in 2003. South Dakota has reported 1,639 cases during 2003 to 2007, which represent of 7% of all cases in the U.S.. This study investigated spatial and temporal patterns of WNV human cases in South Dakota at ZIP Code Tabulation Areas (ZCTA) level and also examined environmental drivers of disease incidence from 2003 to 2007.
ZCTA level WNV human cases were provided by the South Dakota Department of Health. Remotely-sensed environmental variables, including land surface temperature (LST) and normalized difference vegetation index (NDVI), were acquired from the moderate resolution imaging spectroradiometer (MODIS) 8-day composite remote sensing images with 1-km spatial resolution. Precipitation data was estimated from the Tropical Rainfall Measuring Mission (TRMM) dataset with 0.25 degree spatial resolution. The WNV clusters were tested by Local Moran's I statistics with the empirical Bayes smoothed rates. Climate anomaly estimations were based on the 10-year means computed at the ZCTA level.
WNV incidence rates ranged from 6.7-137.7 per million during the study period. 2003 was a significant epidemic year and was followed by 4 endemic years since 2004. Although spatial patterns varied in these years, some persistent clusters were identified on the east side of the Missouri river which may be associated with particular land cover types or ecoregions. LST anomalies and NDVI anomalies in June and July showed a positive association with disease incidence. Precipitation was more variable, but was still correlated with WNV incidence. These results demonstrate that some regions of South Dakota have have a persistently high risk of WNV, and that temporal variability in WNV risk is linked to Spring and Summer weather patterns. These results provide a basis for future efforts to forecast the spatial and temporal patterns of WNV risk in South Dakota. Future research will examine the influences of ecoregion, land cover/use, and vector ecology on the locations of WNV hot spots.

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Seasonal Intermittent Preventive Treatment among children in rural area in Senegal: prevalence of molecular markers of resistance Pfdhfr and Pfdhps.

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Seasonal Intermittent Preventive Treatment among children in rural area in Senegal: prevalence of molecular markers of resistance Pfdhfr and Pfdhps.
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Introduction
The effectiveness of the seasonal intermittent preventive treatment (TPIs) was shown into 2002 with a reduction of the morbidity of 86% (B.Cissé and al.,). By comparing several combinations of antimalarial, Sulfadoxine-Pyriméthamine association with Amodiaquine had appeared most effective but had presented the most undesirable events. However studies showed a high prevalence of mutations in Pfdhfr and Pfdhps genes responsible respectively for resistances to pyriméthamine and sulfadoxine.
Methods
Treatments were delivered to children 3-59 months of age in their homes once per month during the transmission season by community health workers. 33 health workers, each covering about 60 children, were randomized to deliver either SP+AQ, DHA+PQ or SP+PQ. All children had finger prick sample for thick /thin film and filter papers one month after the final round. A molecular analysis determining the prevalence of the molecular markers of resistance to SP was done. This was made with all filter papers corresponding to the positive blades.
Results
1893 children were enrolled, coverage of monthly rounds and compliance with daily doses was similar in all groups, 90% of children received at least 2 monthly doses.
50% of the analysable sample in arm SP+AQ carry triple mutation dhfr (51, 59,108) 40% in arm SP+PQ and 30% in arm DHA+PQ. For dhps gene our results showed that 30% of samples carry simple mutation 437 in arms SP+AQ and DHA+PQ against 22% in arm SP+PQ.
Mutation 540 dhps misses on all the samples.
For the quadruple mutation, our results showed that 25% of sample of arm SP+AQ carry this mutation. It missed in arm SP+PQ
Conclusion
Prevalence of children carrying Pfdhfr and Pfdhps mutations associated with resistance to SP was very low in all groups at the end of the transmission season.

3129

Polymorphism in the viscerotropic gene of the two major *Leishmania* sp. isolated from the soldiers deployed in Middle East

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Leishmaniasis is usually manifested by three different forms cutaneous, visceral and mucocutaneous. *Leishmania major* and *L. tropica* both are responsible for the cutaneous leishmaniasis in the old world. However, in addition to its cutaneous manifestation *L. tropica* has been found to follow different tropism behavior and visceralize in some patients. In order to find the genetic factor behind the tropism behavior, the visceralizing (vis) gene was selected. The study was undertaken to find out the genetic differences in the vis. gene and its possible relationship in the tropic behavior with the two important old world Leishmania sp., *L. major* and *L. tropica* recently isolated from the soldier returning from Iraq and Afghanistan. Primer for the vis. gene region was designed and used to amplify the DNA from the samples, isolated and maintained in our Leishmania Diagnostic Laboratory (LDL). Isozyme analysis was applied to confirm the species diagnosis of the isolated strains before each sample was cryopreserved in the Leishmania Bank at the LDL. The vis gene was cloned from the representative samples of *L. major* and *L. tropica* and sequenced to find the sequence similarity among *L. major* strains and compared with *L. tropica*. Genetic variation in the vis gene sequence of both *L. major* and *L. tropica* was found which indicates that more than one haplotype is present and it is polymorphic in nature. In *L major*, most mutations lead to homologous amino acid changes that are expected to have little or no impact on the conformation of the protein. In contrast, in *L. tropica*, most mutations lead to non-homologous amino acid changes that might affect the conformation of the viscerotropic leishmaniasis antigen. However, it is still not clear if any particular haplotype is responsible for the tropism changes of the *Leishmania* parasite. Additional studies are underway to find a possible link between a vis gene haplotype and change in tropism.

3130

Expression of a Plasmodium falciparum 48/45 gamete surface homology fragment or double domain in Pichia pastoris

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The importance of a transmission blocking vaccine (TBV) to support malaria elimination efforts has recently gained attention. Current efforts have demonstrated that a leading TBV vaccine, identified as Pfs25, induces antibodies in humans that reduce oocyst numbers using a mosquito membrane feeding assay. However our current results indicate that the magnitude and duration of the Pfs25 specific antibody response is insufficient for its success in the field. To overcome this challenge several approaches are being attempted, including protein-protein chemical conjugation, evaluation of various adjuvants, and development of additional TBV candidate antigens. The latter approach has identified potential TBV candidates, in particular two proteins identified as Pfs48/45 and Pfs230. Both of these proteins induce transmission blocking activity, and are members of a family of proteins with a unique cysteine-rich *Plasmodium* gamete surface homology fragment or double domain motif. Numerous efforts, primarily in *Escherichia coli*, to recombinantly produce either of these proteins have achieved limited success in producing functionally folded domain(s). Using a modified *Pichia pastoris* host that overexpresses protein disulfide isomerase, two recombinant double domain protein forms of Pfs48/45 have been developed containing either 9 or 10 cysteines. Antibodies raised in mice to limited quantities of Pfs48/45-9cys double domain recognized non-fixed gametes by an indirect immunofluorescence assay indicating recombinant Pfs48/45-9cys protein mimics parasite protein. Recently we have successfully fermented and partially purified Pfs48/45-10cys double domain protein at approximately 1 mg/L fermentation supernatant with an observable mobility shift upon reduction by Coomassie blue stained SDS-PAGE. At this time, Pfs48/45-10cys protein is being produced using a eukaryotic expression system that facilitates proper disulfide bond formation for biochemical, biophysical and immunological characterization while efforts continue to improve production levels.

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Satellite remote sensing predicts interannual variability in West Nile virus risk in the Northern Great Plains

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West Nile virus (WNV) is a persistent public health problem in the Northern Great Plains (NGP), where incidence has remained high compared to the rest of the United States.. Understanding the spatial and temporal patterns of WNV can help in targeting disease prevention strategies. In particular, there is potential for forecasting disease risk using satellite remote sensing data and geospatial analysis methods. We hypothesized that because of the short growing seasons in the NGP, WNV amplification and subsequent transmission to humans will be related to the timing of spring onset. Our study area encompassed the states of North Dakota, South Dakota, and Nebraska for the years 2004-2008. Remotely-sensed environmental variables, including land surface temperature (LST) and normalized difference vegetation index (NDVI), were acquired from moderate resolution imaging spectroradiometer (MODIS) 8-day composites with 1-km spatial resolution. Cumulative LST and NDVI indices for each MODIS composite period were computed at the county level and expressed as deviations from their long-term means. Temporal patterns of WNV incidence were measured as the

annual relative risk for each county. We found moderately strong correlations between cumulative NDVI and the relative risk of WNV, which peaked at the composite period ending June 17th (r = 0.58) and decreased at earlier and later dates. This result suggests that at a given location, WNV incidence will be higher in years with greater cumulative temperature and precipitation during the spring and early summer months. Cumulative NDVI values in 2009 were low across much of South Dakota and North Dakota, correctly predicting a low risk of WNV transmission. Our work indicates that it is possible to forecast temporal patterns of WNV risk at a regional level using relatively simple metrics derived from satellite imagery. Future research will focus on developing improved metrics of land surface phenology for use in early warning systems, and continuing to test their performance as more years of WNV data become available.

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Geographic Patterns of Plasmodium falciparum Drug Resistance Distinguished by Differential Responses to Amodiaquine and Chloroquine

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Chloroquine resistance (CQR) in *Plasmodium falciparum* originated from at least six foci in South America, Asia and Oceania. Malaria parasites from these regions present variable resistance phenotypes and can be distinguished by point mutations and microsatellite polymorphisms in and near the CQR transporter gene, *pfcrt*, and the multidrug resistance transporter gene, *pfmdr1*. Amodiaquine (AQ) is a pro-drug related to chloroquine (CQ), which is therapeutically effective against most CQ-resistant *P. falciparum* from Africa, but not successful against parasites from large regions of South America. The genetic basis of AQ resistance and the relationship of different *pfcrt* and *pfmdr1* to these drug-resistant phenotypes have been unclear. Using two *P. falciparum* genetic crosses we show that particular *pfcrt* and *pfmdr1* alleles from South America combine to yield greater levels of resistance to monodesethylamodiaquine (MDAQ; the active metabolite of AQ) than CQ, whereas a *pfcrt* allele from Southeast Asia and Africa is linked to greater CQR independent of partner *pfmdr1* allele. Our results, together with i) reported data on PfCRT haplotype distribution; ii) an emerging focus of AQ resistance in Tanzania; and iii) the persistence of 4-aminoquinoline-resistant parasites in large regions of South America, suggest that different histories of drug use on the two continents have driven the selection of distinct *pfcrt* and *pfmdr1* haplotypes. Among these haplotypes, the *pfcrt* allele encoding amino acids SVMNT (codons 72-76) may confer resistance to both CQ and AQ with no noticeable fitness cost for the parasite. We will discuss this hypothesis and its consequences to increased use of AQ and current antimalarial strategies.

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Development DNA Vaccines Expressing the Pre-Membrane and Envelope Proteins of Dengue-1 and Dengue-3

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Dengue virus (DENV) causes the most common arthropod disease in humans. There are four serotypes that provide lifetime immunity against homologous infection, but not against heterologous infection. Instead, pre-existing non-neutralizing antibodies may increase severity leading to dengue hemorrhagic fever, which can progress, into a shock syndrome with potentially lethal complications. Therefore, an effective tetravalent vaccine is required to provide protection against all DENV serotypes simultaneously. Our vaccine candidates express the pre-membrane (prM) and envelope (E) structural proteins because these antigens can elicit neutralizing antibody responses, which are effective mediators of protection against DENV infection. In this study, we constructed two DNA monovalent vaccine candidates against DENV-1 and DENV-3. The prM/env genes were amplified from viral RNA by RT-PCR and cloned into the intermediate vector pcDNA3.1/V5-His©TOPO®. The viral sequences were then subcloned into the eukaryotic expression vector, VR1020. Restriction analysis was performed to verify the insert orientation. Western blot analysis showed that both vectors express the envelope protein in transiently transfected 293T cells. Immunogenecity studies were performed in Balb/c mice to test the immunogenicity of the DENV-1 and DENV-3 DNA vaccine candidates. Indirect immunoflourescence assays (IFA) demonstrated that our vaccines induced high IgG titers. Plaque reduction neutralization tests (PRNT) are in progress to determine if these vectors are capable of inducing neutralizing antibodies. The DENV-1 and DENV-3 vaccine candidates will be incorporated in a tetravalent vaccine formula together with expression vectors against the other 2 DENV serotypes.

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A Recombinant Mosquitocidal Bacterium is Highly Efficacious Against Larvae of Anopheles gambiae

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Current strategies used to control malaria vectors rely primarily on synthetic chemical insecticides in bednets, accompanied in some countries by the use of these chemicals to control adult mosquitoes. Traditionally, larviciding has not been cost-effective in Africa because breeding habitats are often extensive and cryptic. Over the past decade, however, satellite technology and ecological surveys have identified key characteristics of breeding habitats making them easier to find and treat. This is especially true in countries like Kenya, where *Anopheles gambiae* and its sibling species vector malaria. Despite these developments, chemical larvicides remain expensive and detrimental to non-target invertebrates. In addition, as agriculture technologies such as rice cultivation expand, larval breeding has increased substantially. New technologies such as vaccines, new chemical insecticides, and refractory transgenic mosquitoes will not be operational within the next decade. Recently, however, it has been shown that two bacterial larvicides, *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *B. sphaericus* (Bs) can reduce the biting rates of anophelines in Kenya by 90% at a cost of less, making this a potentially useful interim technology. To reduce the cost of bacterial larvicides, we used recombinant technology to combine Bti and Bs mosquitocidal proteins. One recombinant, Bti/BsBin, which produces a large about of the *B. sphaericus* Bin protein in a Bti background, has proven highly effective in laboratory assays. Bti/BsBIn is, respectively, more than 3-fold more effective than Bti, and 15-fold more effective than Bs2362, the commercial strains used in current bacterial insecticides. These results indicate it may be possible to reduce the costs of bacterial larvicides by 50-70% in operational malaria control programs.
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Unique biophysical property of de novo intracellular membrane system in Plasmodium falciparum-infected erythrocyte revealed by Fluorescence Lifetime Microscopy

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P. falciparum infection of human erythrocytes induces dramatic erythrocyte membrane modifications as well as creation of new intracellular membrane systems, including a parasitophorus vacuole membrane (PVM) and Maurer's cleft. Due to the lack of protein trafficking machinery common to other eukaryotic cells, P. falciparum needs to create its own trafficking system. The origins of these membranes and their roles in trafficking are incompletely understood. We have used a cholesterol-rich membrane domain-sensitive fluorophore (Di-4 ANEPPDHQ) and fluorescence lifetime microscopy to investigate properties of the PVM. Fluorescence lifetime of the dye is highly sensitive to the presence of cholesterol-enriched lipid domains. Results showed that the fluorescence lifetime of the dye in parasitized erythrocyte membrane was ~1800-2000 picoseconds (ps), whereas in the PVM and parasite membrane this lifetime was ~1500 ps and ~1250-1300 ps, respectively, significantly lower than in the host erythrocyte membrane. Maurer's cleft-like parasite-derived membranes have a few hundred picoseconds higher lifetime than PVM, showing that properties of these membranes are slightly different from those of the PVM. Further analyses of these lifetime data showed that each membrane has at least two lifetime components suggesting the existence of lateral membrane heterogeneities. When cholesterol was removed from parasitized erythrocytes by methyl-β-cyclodextrin treatment, the fluorescence lifetime of the dye in the erythrocyte membrane and PVM decreased to ~1200 ps and ~1100 ps, respectively, demonstrating that the dependence of fluorescence lifetime on membrane cholesterol content as well as the difference in cholesterol contents between PVM, and erythrocyte membranes. These data suggest that parasite-derived new membranous systems are mixtures of both erythrocyte and parasite-derived lipids and differences in their biophysical properties could be responsible for the complex structures and functions of PVM and Maurer's cleft.

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Functional and structural characterization of the full length var2CSA vaccine candidate

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Pregnancy-associated malaria (PAM) is a serious consequence of *Plasmodium falciparum*-infected erythrocytes sequestration in the placenta through the adhesion to the placental receptor chondroitin sulfate A (CSA). Recent work points to var2CSA, a member of the PfEMP1 family, as the key target for the development of a pregnancy-associated malaria vaccine. However, designing such a prophylactic vaccine has been hindered by the difficulty in identifying regions of var2CSA that could elicit broadly neutralizing and adhesion-blocking antibodies. Var2CSA is a very large protein with an estimated molecular weight of 350 kDa, and can be divided into six cysteine-rich Duffy binding-like domains (DBL). Due to its size and cysteine richness, it has been impossible so far to express a full length var2CSA extracellular region in a heterologous system.
To investigate the role of var2CSA in CSA adhesion, as well as to obtain structural information that could contribute to vaccine optimisation and new therapeutic strategies, we have developed an expression protocol using the human embryonic kidney 293 cell line (HEK293) that yields mg quantities of a full length var2CSA extra cellular region (domains DBL1X to DBL6ε). Data on the functional characterization, as well as the low-resolution structure of the var2CSA in solution obtained recently by small angle X-ray scattering (SAXS) will be presented. This work is providing for the first time a low resolution structural model of a full length PfEMP1 extracellular region and is a major step towards the understanding of the molecular mechanisms involved in CSA adhesion.

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A Real-Time PCR Assay for the Detection of Angiostrongylus costaricensis and A. cantonensis

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Angiostrongylus costaricensis and A. cantonensis are both parasitic nematodes of rodents that cause two different diseases in human beings. A. costaricensis is known to cause abdominal angiostrongyliasis and human infections have been reported in Latin America. In complicated cases, abdominal angiostrongyliasis can result in death due to occlusion or perforation of the intestine. Diagnosis of this illness is made upon the finding of the parasite in tissue samples of cases that require surgery, but typically is based solely on clinical symptoms that can be confused with other conditions. A. cantonensis affects the nervous system and is the most common cause of eosinophilic meningitis in humans. It is the most prevalent in Southeast Asia and islands throughout the Pacific Basin. As for abdominal angiostrongyliasis, diagnosis of A. cantonensis infection is usually based on clinical features of the infection. Since the finding of the immature worms is difficult, demonstration of eosinophils in cerebrospinal fluid aids in the diagnosis. A PCR assay that uses primers based on repeat DNA clones from a genomic library of A. costaricensis has been developed. This assay detects genomic DNA from both Angiostrongylus species based on the amplification of a 219 bp product from A. costaricensis and a 216 bp product from A. cantonensis. Despite the fact that the amplification products are of approximately the same size in both Angiostrongylus species, this does not diminish the potential use of this PCR assay in the clinical setting. These two diseases are so different, that amplification in a PCR test can be interpreted with confidence based on the signs and symptoms of each patient. In addition, the existing evidence of the geographical distribution of human infections with these two parasites does not overlap. Due to the differences of the geographical distribution and clinical manifestations of A. cantonensis and A. costaricensis infections, this PCR assay will prove useful in diagnosing both infections.

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VAR2CSA DBL3X subdomain 3 as vaccine candidate for pregnancy associated malaria (PAM)

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Pregnancy associated malaria (PAM) results from sequestration of *Plasmodium falciparum* infected erythrocytes (IEs) on chondroitin sulfate A (CSA) expressed on placental syncytiotrophoblast during pregnancy. The effects of PAM are most noticeable in primigravidae and include maternal anemia, low birth weight and infant mortality. Multigravidae develop blocking antibodies that bind a preferentially expressed parasite encoded ligand VAR2CSA of *P. falciparum* erythrocyte membrane protein-1 family expressed on the surface of IEs. At least three of the six Duffy-binding-like (DBL) domains of VAR2CSA are known to bind CSA and they are now emerging as primary targets for a PAM vaccine. Due to the polymorphic nature of *var2csa*, domain specific antibodies may not necessarily cross-react with DBL domains of natural *var2csa* expressing parasites. We postulated that cross-reactivity of antibodies may be achieved by expressing the more conserved CSA binding sub-domains of the DBLs. A synthetic codon optimized *dbl3x-s3* gene was expressed in BL21(DE3) *E. coli* cells using a pET24 expression vector. Expressed protein was solubilized from inclusion bodies, refolded and purified by affinity, hydrophobic and ion-exchange column chromatography. The identity and integrity of the refolded, purified DBL3X-S3 protein was established by biochemical and biophysical analyses. Similar to DBL3X expressed in E. coli, FACS analysis showed that DBL3X-S3 bound CHO-K1 cells that express CSA, but did not bind PgsA745-CHO cells that do not express CSA. Rabbit anti-DBL3X-S3 IgG bound homologous FCR3-VAR2CSA IEs, but weakly inhibited their binding to CSA. Similar inhibition of CSA binding was also observed with two maternal field isolates. An assessment of the cross-reactivity of specific FCR3-VAR2CSA IgG to VAR2CSA expressing *P. falciparum* lines is ongoing. If inhibition of CSA binding is additive and limited cross-reactivity of the antibodies is confirmed, then a multi-component vaccine may be developed against PAM provided these in vitro findings translate to clinical protection.

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Quantitative and Temporal Analysis of dsRNA Interference for effective gene silencing in *Aedes aegypti*

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RNA interference (RNAi) techniques have been used to analyze gene functions by reducing target gene expression in various organisms including mosquitoes. In *Aedes aegypti*, RNAi offers a powerful system to investigate not only gene function but also methods of controlling mosquito-borne disease. Among the various methods of RNAi knockdown, intrathoracic injection of ~500 bp-long double-stranded RNA (dsRNA) remains to be the simplest and most amenable approach for application in mosquitoes. To achieve a consistent and effective knockdown, however, the amount of dsRNA injected into the mosquito requires optimization. Here, we evaluated the effectiveness of gene silencing by intrathoracic injection of dsRNAs as a function of varying quantities of dsRNAs

(50, 100, 500, or 820 ng dsRNA/mosquito) using two target genes (cysteine desulfurylase and 1-acylglycerol-3-phosphate acyltransferase) in adult female *Ae. aegypti*. In addition, the duration of dsRNAi was also measured up to 9-days post-injection. Our results showed that intrathoracic injection of 500 or 820 ng dsRNAs per *Ae. aegypti* yielded consistent knockdown for at least 9 days after injection. Therefore, it appears that gene silencing by dsRNAi can persist for a considerable time, which makes most *in vivo* bioassays feasible in *Ae. aegypti*. There appeared to be clear dosage effects of dsRNAi knockdown of target genes in this mosquito, requiring at least 500 ng dsRNAs per mosquito to have effective gene silencing.

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The DHPS Codon 581 Drug-Resistance Allele is Associated with Severe Malaria with Respiratory Distress in Infants

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Respiratory distress is a common presentation of severe malaria in African children. In earlier studies we found that the DHPS codon 581 mutation (c581) was associated with exacerbations of malaria during pregnancy. We hypothesized a more general association between this drug resistance marker and parasite virulence and therefore examined this relationship in children. We selected 15 cases of severe malaria with respiratory distress by WHO criteria, and 42 controls with uncomplicated malaria matched by village and time. Parasite samples from cases contained a higher fraction of c581 resistance alleles (0.47) than those from controls (0.26), although c581 did not predict odds of respiratory distress (unadjusted conditional logistic regression: OR=2.70, p=0.2). Parasite diversity measured by number of MSP-2 alleles trended towards association with odds of respiratory distress (OR=1.19, p=0.1). Parasite density was associated with increased odds of respiratory distress (per 100 parasite increase; OR=1.08, p=0.005). Measured serum sulfa was not associated with case status. In a multivariate model that included c581 fraction, parasite diversity, parasite density, and time; c581 (OR=9.17, p=0.03) and parasite density (OR=1.10, p=0.04) independently predicted increased odds of respiratory distress during malaria. These findings suggest that the DHPS codon 581 drug-resistance allele may be associated with a virulent parasite phenotype in children, even in the absence of sulfadoxine-pyrimethamine treatment.

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Anopheles gambiae densovirus (AgDNV) infection dynamics during mosquito development

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Mosquito densoviruses (DNVs) generally cause severe mortality to mosquito larvae upon infection. In contrast, the Anopheles gambiae densovirus (AgDNV) is completely non-lethal to An. gambiae larvae. We used quantitative PCR to measure the dynamics of AgDNV titer during the infection process in An. gambiae. Viral titers decrease almost 2 orders of magnitude during larval development, reaching levels as low as 5 viral genomes per host genome in the pupal stage. However, after adult emergence, AgDNV levels rapidly increase, with titers reaching approximately 10,000 viral genomes per host genome by the time the adults are 10 days post-emergence. These data suggest that unlike other mosquito DNVs, AgDNV lacks tissue tropism for infection and/or replication in immature mosquito lifestages, but rather replicates preferentially in the tissues of the adult host. The very low virus levels likely contribute to the apparent lack of fitness effects in larval An. gambiae. We hypothesize that infection of the imaginal disks in the pupal stage mediate transtadial transfer of the infection to the adult insect.

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Local spatiotemporal patterns of dengue virus transmission in Iquitos, Peru

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Characterization of the spatial and temporal patterns of dengue virus (DENV) transmission within a region is critical for the design of surveillance and control strategies. Knowledge of the scale of transmission, for instance, will help prioritize the use of limited resources to reduce epidemic transmission. Although dengue infections are known to cluster within households, a key question is at what scale does transmission occur beyond the home. Mark-release-recapture studies indicate that the mosquito vector *Aedes aegypti* disperses short distances (<300 m), predicting clustering within this scale. To examine this question, we carried out Bernoulli space-time analyses of dengue virus infections in a prospective longitudinal cohort of over 4,000 individuals followed from 1999 to 2005 in the Amazonian city of Iquitos, Peru. Infection status was determined by detection of neutralizing antibody to novel serotypes in paired blood samples (seroconversion) taken every 6 months. Date of infection was assigned as the mid-date between the paired samples. We identified serotype-specific spatiotemporal clusters by assigning a maximum radius of 300 m, limiting temporal aggregation to 21 days, and focusing on temporal windows of approximately 3 months. Clustering was detected for all DENV serotypes circulating in Iquitos during the study period, with 1 cluster of DENV-1 in 1999 (p=.005), 1 of DENV-1 in 2002 (p<.001), 3 of DENV-2 in 2002 (p<.037) and 25 DENV-3 in 2002 (p<.045), when DENV-3 was first introduced. The number of cases per cluster ranged from 3 to 34, with radii of 52-300 m and temporal periods ranging from 3 weeks to 3.5 months. These results provide evidence of local clustering of

dengue virus transmission beyond the home. Spatial and temporal clustering of cases over a relatively small geographic area implies that if clusters can be operationally and cost effectively identified, targeted intervention strategies may be an efficient way to utilize limited disease prevention resources.

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Detection of human leptospirosis as a cause of undifferentiated fever by capture ELISA using an M20 strain-derived antigen.

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Background:
Leptospirosis is a zoonotic disease of worldwide distribution and frequently nonspecific symptoms. The diagnosis of leptospirosis is challenging, and serologic confirmation by microagglutination test (MAT) may be time-consuming and difficult in resource-constrained settings. The M20 strain of Leptospira interrogans serogroup Icterohaemorrhagiae is highly prevalent in Perú. In this study, we seek to evaluate the use of antigen derived from the M20 strain in a Mac-ELISA assay, using a total sonicated extract of the genus-specific antigen in the acute and convalescent serum specimens from patients with undifferentiated fever in Perú and Paraguay.
Methods:
Mac-ELISA was used to detect IgM with Leptospira interrogans Icterohaemorrhagiae Serovar Copenhageni strain M20 as the source of antigen. MAT was conducted with a panel of 23 serovars. The cutoff point was determined in 50 sera from healthy subjects using the ROC curve through the Data Statistical Package for the Social Sciences (SPSS). Sera from patients with MAT-confirmed leptospirosis were used as positive controls. MAT was performed on 130 random samples to validate the results.
Results:
Between 2006 and 2007, we collected 6096 acute and convalescent serum samples from 3048 febrile patients with a mean age of 27.6 years from endemic regions of Peru and Paraguay. An ELISA OD cutoff was established at 0.299 with 100% sensitivity and 98% specificity under the ROC curve with CI 95%. From the 3048 acute and 3048 convalescent specimens tested, 154 and 286 were positive by ELISA, respectively. Using the random sampling of 130 MAT-tested sera, we observed a sensitivity of 84% and specificity of 71% in patients during the acute phase by Mac-ELISA for IgM compared with MAT confirmation. Sensitivity increased with convalescent-phase specimens (89%) but with a decrease in specificity (38%).
Conclusions:
An IgM-ELISA derived from the M20 strain of Leptospira interrogans Icterohaemorrhagiae Serovar Copenhageni is sensitive although non-specific for the diagnosis of leptospirosis in Perú and Paraguay. Improved diagnostics for leptospirosis are necessary to improve the management of this disease.

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Trifluoromethyl Derivatives of Reversed Chloroquines have activity against P. falciparum malaria

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Plasmodium falciparum has developed resistance to most of the currently used antimalarial drugs, and there are indications that even the artemisinin-based therapies may be under threat. As a result there is still a need for a pipeline of new antimalarial drugs in development. We have previously synthesized a novel class of quinoline-based antimalarials, named reversed chloroquines (RCQs), consisting of a chloroquine-like portion connected to a chemosensitizer (Reversal Agent, RA). These hybrid molecules have shown excellent activity against P. falciparum malaria, better than chloroquine against both chloroquine sensitive and resistant strains. Here we present the effect of modifications to the quinoline ring system of these RCQs, focusing on the trifluoromethyl group. In in vitro testing against chloroquine sensitve and chloroquine resistant P. falciparum malaria, these new molecules demonstrate good antimalarial activity. There are molecule-specific and substituent position-specific sensitivities to the change of chloro to trifluoromethyl changes, with IC50 values in the sub-10 nanomolar range. Some of the trifluoromethyl-substituted molecules show better activity than their chloro analogues.

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Developing an alternative RNA interference protocol to study druggable targets in filarial worms.

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Diseases caused by fly-borne filaroid nematodes such as *Wuchereria bancrofti*, *Brugia malayi*, and *Onchocerca volvulus* perpetuate socioeconomic instability in developing countries by inflicting crippling morbidity. One reason for the persistence of these diseases is

the limited portfolio of effective drugs, particularly those that are effective against parasite stages other than microfilariae. Drug development for these parasites is complicated by inadequate methodologies to maintain and interrogate parasites *in vitro* through multiple life stages. We tested a novel strategy to trigger RNAi in developing *Brugia malayi* within the mosquito intermediate host. Our initial target gene of interest was the *B. malayi* cathepsin L-like transcript that has been the subject of RNAi silencing and has a role in molting in *O. volvulus*. Compelling preliminary data show that we can acutely suppress expression of this gene in *Brugia* using both short interfering RNA (siRNA) and long double stranded RNA (dsRNA) injected directly into infected mosquitoes. Quantitative RT-PCR confirms that gene knock down is specific and profound, resulting in an 83% decrease in transcript abundance. ***This level of gene knock down has never previously been reported for animal parasitic nematodes.*** Furthermore, cathepsin L-like knock down during the L3 stage results in an aberrant worm phenotype that includes failure to migrate to the head, and therefore significantly reduced potential for transmission. Using this method, we have successfully targeted and suppressed two other genes and therefore demonstrate that other genes are susceptible to this RNAi approach.

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Intrapartum Hepatitis E virus (HEV) infections in rural Bangladesh elicit a pro-inflammatory cytokine response and may contribute to an unrecognized high maternal mortality

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High rates of mortality (up to 40%) have been reported in women infected by HEV in pregnancy, the immunopathology of which is poorly understood. From 2001 to 2006, we enrolled 125,261 reproductive age women into prospective surveillance for pregnancy in a large rural area of northwestern Bangladesh. We identified 67,473 incident pregnancies, outcomes and associated intrapartum morbidities and mortality. 328 maternal deaths were identified; in detailed verbal autopsies by trained physicians, 11% identified acute HEV-like illness in the days preceding death. In a 3% subsample, venous blood was drawn at early and late pregnancy, and at 3 months postpartum. To identify intrapartum HEV infection, we first identified anti-HEV+ individuals at postpartum, working backwards to early pregnancy to detect seroconverters between early pregnancy and postpartum visits. Analysis of 1127 postpartum specimens using an NIH research immunoassay for HEV antibodies (ORF-2) revealed 13.7% anti-HEV seropositivity. Of these, 135 early pregnancy specimens were tested, revealing 72 (53.3%) as seropositive at baseline. Of 63 putative seroconversions (63/1108 = 56 per 1000 PY incidence), 26 had at least a 3x increase in signal-to-cutoff ratio between the early and postpartum samples. Six of these 26 yielded the same result thrice. Cytokine analyses (by MSD) were conducted on 4 subjects with highest antibody titers, compared to 12 uninfected controls and 8 seropositive at baseline women ("non-susceptibles"). Results were consistent with a pro-inflammatory cytokine response (elevated IL-6, IL-10, IL-12 and TNF-a). A pro-inflammatory response to HEV in pregnancy could explain some deleterious effects of infections. We were unable to identify virus in these specimens by RT-PCR. Birth outcomes of 63 seroconverters were live birth (83%), abortion (14%) and miscarriage (3%). We are examining the nutritional profiles of cases and matched controls. HEV infections are occurring in pregnancy in this population in absence of large outbreaks and this prospective data represents the first such evidence. We are planning subsequent large prospective studies to understand the consequences and immunopathology of HEV in pregnancy in these resource poor settings, to provide epidemiologic data for appropriate public health attention to this emerging infectious agent.

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Serosurvey of leptospirosis as a cause of acute undifferentiated fever in Perú, Paraguay, Bolivia, and Ecuador, 2007-2008.

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Background: Leptospirosis is a cosmopolitan disease of major importance. The presentation of leptospirosis varies greatly, ranging from asymptomatic infections to undifferentiated fevers to severe illnesses with pulmonary hemorrhage, kidney injury, and jaundice. Distinguishing leptospirosis from other causes of acute fever is difficult, particularly in resource-constrained settings. In this study, we examine the frequency of leptospirosis among febrile patients presenting for care in a passive surveillance cohort in multiple communities in Perú, Paraguay, Bolivia, and Ecuador from 2007 to 2008.
Methods: Patients with fever >/= 38.0 C for >/= 7 days presenting for care were offered enrollment. Sites were located in Perú (Iquitos, Tumbes, Yurimaguas, Puerto Maldonado, La Merced, Piura, Cusco), Bolivia (Santa Cruz, El Beni, Cochabamba), Paraguay (Boqueron, Asunción), and Ecuador (Guayaquil). Demographic data were recorded and linked with serum specimens. Participants from malaria-endemic areas underwent thick and thin blood smear testing. Acute and convalescent sera were tested by viral culture and PCR for arboviruses and by paired acute and convalescent IgM ELISA for leptospiral, viral, and rickettsial antibodies. The anti-Leptospira IgM ELISA was derived from L. interrogans Icterohaemorrhagiae Serovar Copenhageni M20 strain antigen. A positive ELISA was defined as a fourfold rise in IgM titers or as a single titer >/=1:800.
Results: Paired acute and convalescent sera were obtained from 4,156 participants. Evidence of leptospirosis was highest in sylvatic climates, including 22.4% of acutely febrile patients in Iquitos, 15.2% from Yurimaguas, 14.7% from Puerto Maldonado, and 14.3%

from La Merced. Incidence was also high in the Bolivian lowlands (25.6%). Detection rates were lower from coastal regions (Tumbes, 7.3%; Piura, 8.2%; Guayaquil, 6.2%), the sierra (Cusco, 5.1%), and Paraguay (7.3%). Patients with evidence of leptospirosis tended to be younger and more frequently male than other febrile patients.
Conclusion: Serologic evidence of leptospirosis by ELISA is common among patients with acute fever in Perú, Ecuador, Bolivia, and Paraguay. Rates are higher in the Amazon basin and surrounding area than in the coasts or highlands. Empiric therapy should be considered for ill patients with compatible syndromes in these settings. Improved diagnostics for leptospirosis are necessary to facilitate treatment.

3150

Household Drinking Water Filtration Technologies to Reduce Diarrheal Disease in Developing Countries: A Systematic Review and Meta-Analysis

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Unsafe drinking water and inadequate sanitation and hygiene contribute to over 3.5 billion episodes of diarrhea each year, many of which can be reduced by technologies such as point-of-use (POU) household water treatment. Many household drinking water treatment technologies are available for use in areas with unreliable water supplies including: chlorination, flocculation-disinfection, solar disinfection, filtration and boiling, along with safe storage. The most appropriate choice for a specific intervention largely depends on local socio-cultural practices and perceptions. Filtration technologies are an attractive option as they are often effective, cost-efficient and sustainable. In order to further evaluate and characterize diarrheal disease reductions, a systematic review and meta-analysis of the literature on household POU filtration in developing countries was performed. In total, 14 intervention studies documenting reductions of endemic diarrheal disease incidence in all ages and in children under five years old were selected for analysis. The systematic review revealed that POU filtration reduced diarrheal disease in all cases. Results from the meta-analysis suggest significant funnel plot asymmetry. There was evidence of bias from colinearity in research attributes, lack of blinding within study protocols, or publication bias within the literature. Random-effects meta-regression analyses suggest ceramic candle type filters were more effective than other filter types such as porous ceramic pot filters or biosand filters. Additional results from the meta-regression found more significant reductions in diarrheal disease for children two to four years of age (2 studies) and in studies performed in Sub-Saharan Africa (2 studies). Results suggest that future research on the health benefits of POU filtration should further characterize the impact on children less than five years of age, especially in populations of Sub-Saharan Africa. Overall, the results of this meta-analysis document the effectiveness of household water filtration in reducing diarrheal disease.

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Growth inhibitory activity of antibodies generated to vaccine candidate *Plasmodium falciparum* Merozoite Surface Protein-1 (MSP-1_{42}) Administered Intramuscularly with GSK Biologicals' Adjuvant System AS01B in Healthy Malaria-Naïve Adults

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At WRAIR, the vaccine antigen Merozoite Surface Protein-1 (MSP-1) has been under development for the past 15 years. We have previously reported results from two separate Phase 1a studies of the 42 kDa C-terminal fragment of MSP-1 (MSP-1_{42}): one in which the MSP-1_{42} antigen representing the 3D7 allele was formulated in GSK's Adjuvant System AS02A and alternatively, one in which the FVO allele of MSP-1_{42} was formulated in AS01B. Antibody results from preclinical rabbit studies have demonstrated the improved immunogenicity of the FVO allele of MSP-1_{42}, and when evaluated in humans using *Luminex*™ technology, the vaccine candidate MSP-1_{42} FVO/AS01B elicited higher titers than MSP-1_{42} 3D7/AS02A to both FVO and CAMP alleles of MSP-1_{42}, the two alleles found in 90% of field isolates in Western Kenya. To better assess the potential functionality of this antibody response we conducted growth inhibition assays (GIA) with serum from volunteers immunized with MSP-1_{42} FVO/AS01B in two types of GIAs: one with 20% serum (v/v) and the other using purified immunoglobulin at concentrations of 2, 4 and 8 mg/ mL. For the serum assay, the inhibitory activity of antibodies against homologous parasites ranged from 0-24% and was not statistically different from the homologous inhibition obtained with serum from volunteers vaccinated with MSP-1_{42} 3D7/AS02A. There was little inhibition against heterologous 3D7 parasites. For the purified immunoglobulin, a dose dependent titration was seen for inhibition against homologous FVO parasites with 4 of 18 volunteers having inhibitory activities >60% at either 4 mg/mL or 8 mg/mL. While the ELISA titers clearly supported the preclinical data from rabbits that the FVO allele is more immunogenic and cross-reactive than the 3D7 MSP-1 allele, the GIA data obtained from U.S. naïve volunteers does not indicate any significant difference between the two. To evaluate whether antibody titers to homologous and heterologous alleles are more predictive of clinical efficacy than the *in vitro* GIA will require a Phase 2b study in a malaria endemic area.

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Membrane Disruption during Calpain-Mediated Egress of Apicomplexan Parasites

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Apicomplexan parasites are obligate intracellular pathogens that exhibit complex life cycles with distinct sexual and asexual phases. The process of replication within the asexual phase of the life cycle occurs within a specialized parasitophorous vacuole (PV) to yield multiple daughter parasites. These daughter parasites must escape from both the PV and the host cell plasma membrane (PM) in order to reinvade uninfected cells and maintain infection. Apicomplexan egress from host cells is an explosive event involving both calcium and proteases, though the precise mechanism is unknown. Our group has recently reported that apicomplexan parasites co-opt host calpain proteases to allow for efficient egress from the host cell. Here we characterize the mechanism of calpain-mediated parasite egress via live cell microscopy of induced egress via ionophore treatment in T. gondii and natural egress in both P. falciparum and T. gondii. Fluorescent markers within the PV space and host cell cytoplasm allow for elucidation of the sequence of membrane disruption during egress in the presence and absence of calpain. P. falciparum and T. gondii breakage of the host cell PM is blocked by calpain immunodepletion or genetic depletion of calpain activity, respectively, though PV membrane disruption is unchanged. The parasite protein TgPLP1 has also been implicated in escape from the PV and PM. Live cell microscopy of induced egress of PLP1-KO T. gondii tachyzoites from CAPNS-/- host cells indicates a block in both PV and PM disruption, which is restored by genetic complementation of both calpain and TgPLP1. Further studies are underway to elucidate the precise mechanism of parasite egress, though we suggest a model in which a calcium signal triggered at late schizogony activates parasite PLP1, which associates with the PV and allows calcium flux into the host cell. Activated host cell calpain relocalizes to the PM to cleave cytoskeletal proteins and facilitate parasite egress.

3153

Neutralizing and non-neutralizing monoclonal antibodies against dengue virus E protein derived from a naturally infected patient

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Though antibodies produced during dengue virus infection provide homotypic immunity, prior infection or circulating maternal antibodies can mediate a non-protective antibody response. It is thought that these subsequent heterotypic infections can intensify the course of disease. Therefore, naturally occurring human monoclonal antibodies may help us understand the protective and pathogenic roles of the humoral immune system in dengue virus infection. Epstein-Barr Virus (EBV) transformation of B cells isolated from peripheral blood from a human subject with previous dengue infection was performed. B cell cultures were screened by ELISA for antibodies to dengue (DENV) envelope (E) protein and cloned by limiting dilution. Human monoclonal antibodies (hMAbs) were purified and binding specificity to E protein was verified by Western Blot, ELISA, and biolayer interferometry. Neutralization, affinity binding and enhancement assays were conducted on the three IgG1 hMAbs generated: 2.3D, 3.6D and 4.8A. All three hMAbs bound to at least two of the four DENV serotypes and all were able to enhance entry of DENV-1 into K562 hematopoietic cells. Only 4.8A demonstrated prominent neutralization against DENV-1 and -3. These hMAbs were successfully generated by EBV transformation of B cells from a patient at least two years after naturally acquired DENV infection. These antibodies show different patterns of cross-reactivity, neutralizing and enhancement activity.

3154

Macrophage Migration Inhibitory Factor Promoter Polymorphism is Associated with Anemia in *Plasmodium vivax* Infection

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Over the past decade, malaria has been considerate a primarily inflammatory cytokine-driven disease. Because most studies have focused on *Plasmodium falciparum*-associated neurological syndrome, little is known about *Plasmodium vivax*, the most prevalent cause of malaria outside Africa. Here, we investigated the influence of genetic variation within the promoter region of tumor necrosis factor-alpha gene (TNF-α, -308 G/A and -1031 T/C) and macrophage migration inhibitory factor gene (MIF, 173G/C and -794CATT5-8) on susceptibility to anemia and/or thrombocytopenia associated with *P.vivax* infection. For that, we included patients (aged 20-68 years, n=187) who had acquired *P.vivax* infection in the Brazilian Amazon area. The only significant association was found between MIF-173 genotypic variants and anemia. In addition, we found that *P.vivax* infection is characterized by the presence

of plasma circulating microparticles, which seems to be produced by numerous cell types, including platelets and erythrocytes. (Supported by CNPq, PAPES/FIOCRUZ and FAPEMIG)

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Effect of albendazole treatment on gene expression in *Brugia malayi*.

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In recent years, resistance to benzimidazole anthelmintics, including albendazole (ABZ) has reached alarming levels in numerous important nematode parasites of livestock. Mass treatment programs for elimination of lymphatic filariasis in human populations using ABZ may place similar types of selective forces for anthelmintic resistance. Therefore, there exists a need to improve our understanding of the molecular mechanisms underlying resistance to ABZ in *Brugia malayi* so that molecular assays can be developed to monitor the emergence of resistance. Since filarioid nematodes are genetically diverse, we hypothesize that gene expression levels will vary between worms that demonstrate differential susceptibility to ABZ, as well as between treated and untreated worms. Thus, we may begin to understand which genes regulate ABZ action on *B. malayi*. In order to detect variations in gene expression, we surgically-transplanted adult female *B. malayi* worms into gerbils and treated them with ABZ. Worms were isolated from gerbils 21 or 28 days later and separated into two groups based on their motility scores: 1) worms which maintained full motility (tolerated ABZ), and 2) worms with reduced motility, which would die soon thereafter. RNA from the two sets of worms, along with untreated control worms, was then submitted for microarray analysis. Arrays were hybridized with Cy3 and Cy5 dye-labeled cDNA from the two different motility groups and controls. Both biological and technical replicates were included in the experimental design. After normalization for probe and background intensity, the relative intensity of hybridized cDNA was determined for each spot. 207 genes were upregulated 5-fold or more while 125 genes were down-regulated 5-fold or more ($P<0.05$). Genes were also dysregulated between the treatment and control groups. Gene ontological analysis is currently in progress, but dysregulated genes include a cation-transporting ATPase, DNA polymerase I and two proteins containing Zinc finger domains.

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Quantification of the in vitro growth dynamics of multi-clone Plasmodium falciparum infections

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Plasmodium falciparum infections do not usually consist of a single, genetically distinct clone; rather, humans often are co-infected with multiple parasite clones. The presence of multiple genotypes could influence the spread of drug resistance and parasite virulence. We have investigated the growth dynamics of pairs of genetically distinct *P. falciparum* clones *in vitro*. In two-clone infections, the relative growth advantage of one of the clones drives the other to sub-detectable levels over time. Often, the outcome of the pair-wise growth assay can be predicted by the growth rates of each individual clone; however, occasionally the outcome of two-clone infections does not reflect single-clone growth characteristics, indicating some form of interaction between different clones. Previous methods were limited to observing shifts in clone composition by measuring relative amounts of each parasite. Here we demonstrate a quantitative PCR based method for tracking growth in multi-clone infections. Mixtures initially containing equal numbers of genetically distinct parasites were co-cultured *in vitro*, and the number of each parasite quantified over several erythrocytic cycles. The effect of varying parasite density and nutrient conditions was also investigated. Additionally, mixtures of chloroquine-sensitive and resistant parasites were subjected to drug pressure to monitor the effect of drug on each of the parasite populations. This approach will provide greater insight into complex growth dynamics in multi-clonal infections that could aid in the implementation of novel control strategies.

3157

Clean Water a Top Concern for Rural Hondurans: A Needs Assessment Survey

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OBJECTIVES:
To describe the results of a June 2008 needs assessment survey administered to people in the Yoro area of Honduras.
METHODS:
A needs assessment survey based on key health indicators was designed and randomly administered to people in the Yoro area of Honduras. Data on demographics, access to health care, environmental health pressures and perceived health needs were collected.
RESULTS:
There were 70 respondents. The mean age was 38. 49 were women and 21 were men. Among the health indicators queried, water source and methods for water purification were examined. One question asked was, “What is the primary source of drinking water used by your family?” Answers to this question included: water from plumbing (48 respondents), natural fountain (4), well (6), public

water fountain (1), water truck tank (0), river or stream (10) and other (1). An additional question asked was, "How do you treat the water to make it safe to drink?" Answers included: boil (20), chlorinate (25), filter (1), let it sit (0), no treatment (14), multiple methods (7) and unreported (3). Of 70 respondents, 53 people (75.7%) treated their water with at least one purification method. Of those who use water from plumbing, 38 of 48 (79.2%) treated their water with at least one method. Of those whose primary source of drinking water was a river or stream, 7 of 10 (70%) reported treating their water. Additionally, survey respondents were asked to identify the health problems about which they were most concerned, with the 2 most common responses being water sanitation and nutrition.
CONCLUSIONS:
The sources of drinking water among respondents varied widely. However, regardless of source, most people attempt purification and perceive drinking water sanitation as a major health problem. These data will help inform future public health interventions in this region.

3158

Novel Pre-erythroytic Stage Vaccines Against Malaria

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One mechanism by which *Plasmodium* species evade the immune system is suppressing the expression of costimulatory molecules on antigen-presenting cells. We have genetically engineered novel vaccine prototypes to overcome this mechanism of immune evasion. The vaccines consist of a CD4 T cell epitope from *P. yoelii* CS protein built on an anti-DEC205 Ig scaffold and expressing the costimulatory domains of CD80 or interleukin-4. The costimulatory domains expressed by the chimeras are required for Th1 and Th2 cell differentiation, respectively, whilst the DEC 205 Ab specificity provides binding to the dendritic cells. Each chimeric vaccine, one designed to promote Th1 (PyTh1) and the other Th2 (PyTh2) differentiation, binds to the DEC205 receptor expressed on transfected cells. The costimulatory domains are fully functional as indicated by phosphorylation of T cell-intracellular signaling modules, PI-3K and STAT6. Administration of these chimeras to BALB/c mice followed by sporozoite challenge significantly reduced the burden of liver stage parasites (88.5±9.8% for PyTh1-immunized mice and 90.7±7.7% for PyTh2-immunized mice) compared to non-immunized mice. The results indicate that these vaccine platforms are able to provide costimulation to T cells and may represent a suitable approach to overcome the immune suppression induced by malaria parasites and thereby to elicit protective immunity.

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A new direct fluorescent antibody test for the diagnosis of falciparum malaria in blood films

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Conventional stained blood film microscopy for malaria diagnosis remains one of the most tedious and time-consuming activities in parasitology. In expert hands the technique is highly sensitive but for the vast majority of laboratories accuracy of diagnosis is little better than 70%, particularly at low parasitaemia levels. The introduction of lateral flow immunochromatography rapid tests (RDTs) over the past 15 years has gone some way towards addressing this problem but again these tests encounter sensitivity problems at low parasitaemia levels. The issue of sensitivity can be overcome by multiplex PCR assay systems but at the present time this technology is restricted to a few major reference laboratories.
One sensitive immunological method that has not been applied to malaria diagnosis is direct immunofluorescence (DFA), mainly because the high cost and maintenance of UV microscopes has made their routine use impractical and prohibitively expensive. We report here the development of a direct immunofluorescence reagent (RAPIMAL FA) for staining P. falciparum in blood films. This development coincides with the availability of a new generation of low cost, mains, car battery, or solar-powered LED fluorescence microscopes or adapters which put this technology in reach of almost any laboratory or field station. One such adapter allows the conversion of any light microscope into a fluorescent microscope in a matter of minutes.
The reagent is a P. falciparum specific monoclonal antibody conjugated to fluorescein isothiocyanate (FITC). Malaria-infected red blood cells appear as bright apple-green "beacons" against a dark reddish-brown background. This reagent has been used to detect infected RBCs (iRBCs) in blood samples showing a wide range (as low as <0.01% to as high as 9%) of parasitaemia.
Direct FA screening offers a number of advantages over conventional microscopy:
• The apple-green parasite-infected red blood cells are easily visible against a dark background
• The background greatly reduces eye fatigue and makes it relatively simple to scan a broad area of the blood smear using x40 or x60 objectives prior to using higher magnification.
• Monoclonal antibody specificity simplifies the diagnosis of falciparum malaria and reduces the level of false positives resulting from staining artefacts
• Increased detection at low parasitaemia levels (< 0.01%) overcomes the sensitivity issues common to RDTs

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Dynamic Measurement of P. falciparum-infected Erythrocyte and Human Endothelial Cell Interactions

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The adhesive interactions between P. falciparum-infected erythrocytes (IRBC) and microvascular endothelium are critical in the development of severe malaria. The most commonly used methods to study this process are various forms of cell adhesion assays that estimate bulk adhesion without measuring the underlying biophysical forces that are involved. In this study, we used atomic force microscopy (AFM) to analyze the interaction between IRBC and human dermal microvascular endothelial cells (HDMEC) at the single cell level. A single live IRBC, attached to the end of the cantilever, served as a functionalized probe that monitored the IRBC-endothelial cell interaction in real time. Our results show that the initial tethering of IRBC-endothelial cell conjugates involved a rupture force of 225.7 ± 54.9 pN (mean ± SEM, n=23) that was composed of 4.66 ± 0.55 rupture events. In comparison, uninfected erythrocytes displayed a rupture force of 42.7 ± 7.7 pN (n=7), and involved 0.89 ± 0.24 rupture events. For a given IRBC, the force of detachment remained constant for up to 20 sequential contacts and for up to 5 different endothelial cells in a monolayer. The detachment force was reduced to baseline levels by an anti-CD36 but not an anti-ICAM-1 antibody. Interestingly, detachment force and the number of rupture events increased with time as the IRBC was left in contact with the endothelium, so that by 300 seconds the force of detachment had increased from 101.7 ± 17.7 to 756.4 ± 137.8 pN (n=4, p = 0.017), and the number of rupture events had increased from 4.4 ± 1.4 to 27.9 ± 8.6 (p = 0.057). The time-dependent increase in the strength of adhesion was inhibited by the Src family kinase inhibitor PP1 as well as the actin polymerization inhibitor cytochalasin D. These results provide important new insight into the regulation of endothelial cell responses by P. falciparum.

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Causal mechanisms of hand contamination with fecal indicator bacteria among mothers in Dar es Salaam, Tanzania

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Despite the recent surge in interest among international health organizations to promote hand hygiene, rigorous evaluation of handwashing interventions is hampered by the lack of reliable indicators that can be used to measure adherence to hand hygiene behaviors in the field. Many studies to date have relied on self-reported data, which is often biased due to social desirability. Measuring levels of fecal indicator bacteria (FIB) on hands has begun to be used as an indicator of hand cleanliness, but lack of knowledge regarding how concentrations of FIB on hands change over time, and how daily activities affect levels of FIB, can render results difficult to interpret.
In order to characterize the changes in FIB concentrations on hands attributable to typical daily activities, and to better understand temporal dynamics of FIB on hands, we conducted an observational study, along with hand rinse sampling, among 119 mothers in Dar es Salaam. One cohort of 22 mothers was observed by enumerators over an 8-hour period while they performed their daily activities. Enumerators documented each activity performed and obtained a hand rinse sample from the mother every 2 hours. Members of a second cohort of 97 mothers were each asked to carry out one of the following activities: sweeping, food preparation, dish washing, bathing, cleaning the toilet, defecation, urination, or cleaning up after a child who had defecated. Hand rinse samples were obtained before and after each activity to determine the change in FIB concentration on hands. A control group was enrolled to document changes in FIB levels over the same timeframe, during which each mother held her hands still on a clean paper towel. All hand rinse samples were processed by membrane filtration to enumerate *E. coli* and fecal streptococci. The results from this study provide quantitative data on the effect of specific activities on hand contamination, as well as valuable insights regarding the types of interventions that would be most effective in reducing hand contamination among mothers in Dar es Salaam.

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The role of bats as potential reservoirs for *Leishmania* in Mexico

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The role of bats as reservoirs for many zoonoses has been a subject for discussion in the latest years. Bats are reservoirs of 66 species of virus and in recent years have been found to play a leading role between zoonotic diseases and human cases, as has been reported for Ebola and Nipah virus. In the case of leishmaniasis, although they have not been confirmed as reservoirs yet, there is evidence that sand flies can feed on bat wings. One bat species, *Carollia perspicillata*, has been found naturally infected with *Leishmania chagasi* in Venezuela. In a previous study, using biotic interaction networks, species that could potentially act as *Leishmania* reservoirs in Mexico were ranked, based on their geographic coincidence with sand flies. As a result, the list of suspected reservoirs had a high representation of bats. With the purpose of addressing the role of bats as potential reservoirs for *Leishmania* and continue to include them in the predicting models, we conducted a field validation sampling for bats in the states of Chiapas and Tabasco, in Mexico. In this locations cases of leismaniasis are known to occur. As a result, 71 bats were captured and tested for parasites in skin, liver and

heart using the polimerase chain reaction. Nine individuals belonging to 7 species were positive. Individuals were asymptomatic except for one *Artibeus intermedius* that showed skin lesions.
Once species with *Leishmania* parasites were identified, an ecological description was made for each species in order to define patterns or characteristics that make bats suitable reservoirs for *Leishmania*.
Studies on *Leishmania* reservoirs usually are focused in few taxonomical groups or domestic animals, however, the role of other taxa such as Chiroptera must be defined since their ecological characteristics make them efficient dispersors for many diseases and can be a key factor in the process of domiciliation. The role of bats as *Leishmania* reservoirs must be a prioritary field of study.

3164

Rapid serological assay for determining Strongyloides infection using diffraction-based optical biosensors

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Strongyloidiasis is a chronic parasitic infection caused by the intestinal nematode *Strongyloides stercoralis*. Infection is acquired through contact with feces contaminated soil or water and it is estimated that 30-100 million people worldwide are currently infected. Infection can persist for decades because of the ability of *S. stercoralis* to replicate within the host and can go undetected since infected individuals are generally asymptomatic. Immunosuppression in these patients can result in hyperinfection and disseminated disease with an associated mortality of over 80%. Current serological assays use heterologous Strongyloides antigen to detect serum antibodies by ELISA, which is both time consuming and prone to false positive results due to cross-reactivity to other helminth infections. Here, we describe a rapid method of determining Strongyloides infection using a recombinant antigen from L3-stage larvae to capture serum antibodies from only 10 µL of patient serum. Antibody levels were determined using a novel, automated, diffraction-based optical biosensor technology and generated results in approximately 40 minutes. Analysis of gold standard (stool positive)/ELISA positive Strongyloides samples and samples from healthy individuals and patients with other parasitic infections demonstrated an assay sensitivity of 82% and specificity of 97%. The ability to generate quick results and good assay performance make this test conducive to possible future point-of-care use where rapid diagnosis is needed.

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***In vitro* Antimicrobial Evaluation of *Phyllanthus fraternus* using the Microdilution Method**

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This study focussed on the antimicrobial potentials of the extracts of *Phyllanthus fraternus* (Euphorbiaceae), a medicinal plant, that has undergone extensive phytochemical research spanning over four decades using microbes such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* as test organisms.
A known amount of dried *P. fraternus* (Whole plant) was boiled in water and the aqueous extract obtained freeze-dried to obtain a fine powder. An initial stock concentration of 32mg/ml of the powder was prepared using sterile distilled water and serially diluted two-fold. A known volume of the extract was mixed with a known amount of the test organism in a 96 microplate and incubated for 24 hours. After the incubation period, 40µl of 0.2 mg/ml INT (Iodonitrotratazoliumviolet) dissolved in sterile distilled water was added to each of the wells and again incubated for 60-120 minutes. The results indicated a strong antimicrobial activity of the extracts against all the test microbes with the MIC values ranging from 0.5 to 2mg/ml. Extract from the plant also showed better anti-bacterial activity than the reference antibiotic (penicillin-streptomycin). Thus, *P. fraternus* has the potential of being developed into a powerful antibiotic against infections caused by these test organisms.

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Treatment Patterns for febrile illness in two peripheral health facility settings in rural Ghana.

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Background: Malaria is known to be frequently over-diagnosed with resultant over-prescription of anti-malarials. Difficulty in differentiating between the different causes of febrile illness has been cited as a contributory factor to the over-diagnosis of malaria in African settings with very little laboratory and other diagnostic support. Even where laboratory support exists it has been found that results are often ignored by clinicians. Several other factors may be linked to treatments given by clinicians.
Setting: Four clinics in rural Ghana; one where miscroscopy exists, and three others where diagnosis of malaria is clinical (made without tests).
Methods: A Randomised Trial. Patients for whom clinicians suspected malaria were randomised either to a RDT or current diagnostic method (microscopy or clinical diagnosis) in the two settings. The relationship between anti-malarial treatment and various factors

were explored.
Results: Results of the main trial are reported elsewhere. Of a total of 8572 patient consultations in the two settings, 4613 (53.8%) thought they had malaria while 437 (5.1%) said they did not. Anti-malarial treatment given by clinicians did not appear to be influenced by the patient's perception of his/her diagnosis. 263(60.2%) of the patients who did not think they have malaria received an anti-malarial as did 440 (71.3%) who were certain they had malaria. Of 514 patients who had taken an anti-malarial before the visit, 179 (34.8%) had taken ACTs while 103 (20.0%) took chloroquine. Those who had not taken an ACT were more likely to receive an anti-malarial than those who had taken an ACT (OR 1.98, 95% CI 1.31 to 2.98; p<0.001) No diagnosis was written for 188 (71.2%) of the patients. 140 (6.2%) who did not have a diagnosis of malaria still received an anti-malarial.
Conclusions: Targeting of anti-malarials to those who need them remains an issue that needs to be addressed in many African settings. Where microscopy exists and should lead to improved targeting, prescribing patterns do not appear to differ much from other settings where diagnosis is mainly clinical. Other methods of improving diagnosis need to be considered.
Trial Registration: ClinicalTrials.gov NCT00493922.

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Severe adverse events following 17DD vaccine in area with vaccine intensification in South Region of the Brazil, 2008-2009

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Background: Yellow fever vaccine (YFV) is the unique effictive control and prevention measure. Severe adverse events following YFV (SAE) has been described with 3-8 cases/million doses YFV distributed. The main objective for this study was to evaluate the frequency and to describe the SAE in area with vaccine intensification in South region of the Brazil. Methods: We used three case definitions for SAE. YFV associated-viscerotropic disease (YEL-AVD) for syndrome like wild YF infection, YFV associated-neurotropic disease (YEL-AND) for presentations as viral meningitis, and YFV associated-auto-immune neurological disease (YEL-AAiD) for peripheric or central nervous system desmielinization manifestations. We conducted this study with people that received the YFV from December 2008 to April 2009. We included patients from the National Information System for Adverse events following Vaccination (SI-EAPV) and active search in five reference hospitals. Results: Seventy seven patients were identified, 75 (97%) were first-time vaccinee, 40 (52%) were male, 20 (26%) presents YEL-AVD, 50 (65%) YEL-AND and seven (9%) YEL-AiAD, respectively, with risk of 8,2; 20,7 and 2,8 per 1000,000 YFVadministered doses. In YEL-AVD, the median age was 44 (3-76) years-old, the median onset symptoms was 4(1-15) days after YFV, the mainly symptoms were fever 17(85%), nausea/vomiting 12(60%) and headache nine (45%). In YEL-AND, the median age was 16 (0-76) years-old, the median onset symptoms was 7(0-30) days after YFV, the mainly symptoms were headache 48(98%), fever 38(76%) and nausea/vomiting 35(75%). In YEL-AiA, the median age was 29 (10-46) years-old, the median onset symptoms was 4(0-27) days after YFV, the mainly symptoms were weakness seven (100%) and paresthesis four (57%). All five deaths (case fatality ratio=6.5%) occurred in the YEL-AVD. Conclusion: There was high frequency of SAE. Higher frequency happened in YEL-AND. Death occurred in viscerotropic disease. We suggest the developing of others studies to identify risk factors associated with SAE.

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Chagas Serosurvey Near Autochthonous Human Case in Louisiana, USA

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Although rare in the United States, there have been several reports of autochthonous transmission of Trypanosoma cruzi. In June 2006, we identified a human case of domestically-transmitted T. cruzi in southern Louisiana. From September 2008 to November 2009 we evaluated the localized risk of human T. cruzi infection by conducting a serological survey, environmental assessments of residences, and entomological investigations. Residents of households in close proximity to the human case were visited and offered rapid Chagas testing and a perimeter search for Triatoma sanguisuga, the predominant local triatomine bug species. In addition, household demographics were collected and properties assessed to determine risks associated with the presence of T. sanguisuga. Sixty-eight households were enrolled in the study and 124 individuals were tested for T. cruzi infection using the Chagas Stat-Pack Assay (Chembio Diagnostic Systems, Inc. Medford, NY). Serological results were confirmed with a Chagatest ELISA recombinant V 3.0 (Weiner Lab, Rosario, Argentina), modified for use with finger stick blood on filter paper (see Late Breaker by Londono et al). No study participant tested positive for parasite antibodies (exact binomial 95%-C.I. 0%-3.3%). Ninety-two whole and 4 partial T. sanguisuga insects were collected from 11 enrolled households. Prevalence of T. cruzi infection among triatomine bugs tested (n=88)

was 69.3% (95%-C.I. 59.3%-78.7%). Systematic differences between environmental characteristics of households or behavioral characteristics of homeowners who reported presence of T. sanguisuga insects compared with those who did not are currently being analyzed. While the human risk for autochthonous Chagas disease in the studied population seems low, large numbers of infected T. sanguisuga indicate a substantial latent risk for T. cruzi transmission.

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Pathogenesis Studies of the Puerto Rico WNV Isolates

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West Nile Virus (WNV) was first isolated from a chicken and mosquitoes in Puerto Rico (PR) in 2007. That same year, three WNV-positive asymptomatic blood donors were identified in PR however confirmed human cases of symptomatic WNV had not been reported. Hyperendemicity of the dengue viruses (DENVs) creating a cross protective immunity to WNV or attenuated WNV strain could explain the paucity of symptomatic cases in PR. To test the later, we developed an animal model to study the pathogenesis of the PR WNV isolates in Balb/c mice. Mice were inoculated with the PR isolates PR423 (mosquito) and 20WH (chicken), and the parental strain, NY99, which were passaged in two different cell lines, C6/36 and Vero. Survival rates for 20WH, PR423, and NY99 were 50%, 30%, and 20%, when passaged in C6/36 cells and 70%, 40%, and 30% when passaged in Vero cells. The survival rate was significantly greater with WNV isolate 20WH when compared to NY99 (log rank, p=0.04). Although there was no statistically significant difference in survival between NY99 and PR423 (log rank, p=0.98), the average survival time (AST) was significantly higher in PR423 than in NY99 (t test, p=0.013), when both strains were passaged in C6/36 cells. We also compared mouse survival based on cell lines (C6/36 vs. Vero) and no statistical differences were observed. However, AST was significantly higher in C6/36-derived PR423 when compared to Vero-derived PR423 (t-test p<0.001). These results suggest that the PR isolate 20WH appears to be attenuated. This finding is consistent with previous observations, in which smaller plaque morphology was observed with 20WH when compared to NY99 suggesting attenuation (Hunsperger, unpublished results). In addition, we found that PR423 is a pathogenic strain that causes faster mortality than NY99, when passaged in C6/36 cells. The data also suggests that C6/36-derived viruses are not more pathogenic than viruses grown in Vero cells. Viral load determinations by real time RT/PCR are in progress and will be presented.

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A risk reduction assessment of Vibrio and Salmonella in contaminated drinking water after a contact disinfectant point-of-use treatment device

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IWA HRWM 2009
Coulliette Abstract
The possible transmission of *Vibrio cholera* and *Salmonella enteric* serovar *Typhi* in developing countries through drinking water illustrates a need to evaluate point-of-use (POU) devices. A risk assessment approach was used to evaluate the reduction of *V. mimicus* and *S. typhimurium* concentrations via the HaloPure® halogenated POU. Well water was seeded with and without domestic raw sewage (10%), *S. typhimurium*, and *V. mimicus*. For *Vibrio,* the risk was based on 1, 10, and 100 infected persons contributing their pathogens directly to the sewage leaking into the water source. For *Salmonella*, the risk was based on sewage contributions in a community of 100,000 with 1, 10, and 100 infected persons with acute salmonellosis (1). The estimated volume of drinking water ingested was 800 ml/day (2). The chlorine HaloPure® unit illustrated an average 4.9 $\log_{10}$ reduction of *S. typhimurium* and 2.58 $\log_{10}$ reduction of *V. mimicus*. The estimated *Salmonella* after POU treatment would zero when 1 or 10 person(s) were infected and negligible with 100 people infected. *Vibrio* disinfection rates following POU treatment for 1, 10, and 100 infected persons in a community would contribute 2.95×10^5, 2.95×10^6, and 2.95×10^7 *Vibrio* per L. For *Vibrio*, if 10 or more people are infected, the HaloPure® chlorine canister would not sufficiently reduce the threat of cholera. Under the given conditions with the assumptions used in this study and using the Beta-Poisson models established for *Salmonella* and *Vibrio* (2), there would be a daily probability of infection of 5.70×10^1 to 2.43×10^3 per 10,000 and 7.68×10^2 to 9.81×10^2 per 1,000, respectively, without POU treatment and 6.62×10^{-4} to 6.6×10^{-2} per 10,000 and 1.87×10^1 to 5.62×10^2 per 1,000 and, respectively with POU treatment, respectively. References: (1) Kinde, H. and Atwill, E.R. (2000). "*Salmonella* in sewage effluent raises ecological and food-safety concerns." *California Agriculture*. 54(5):62-68. (2) Haas, C.N., J.B. Rose, and C.P. Gerba (1999). Quantitative Microbial Risk Assessment. John Wiley & Sons, Inc. New York, NY.

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MOLECULAR DIAGNOSTIC AND CHARACTERIZATION OF Trypanosoma cruzi IN Triatoma infestans COLLECTED IN La Joya, PERU.

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Recent multinational efforts to stop active transmission of Chagas disease have proven effective in Southern Cone countries, however active transmission still occurs in other areas of South America. We conducted a study in the community of La Joya, in Arequipa, Peru to determine the presence and lineages of Trypanosoma cruzi in Triatoma infestans. Blood meals from 43 T. infestans collected prior to insecticide spraying were screened using primer sets S35-S36 (kDNA minicircle variable regions) and Tcz1-Tcz2 (195 bp nuclear repeat). Positive samples were then typed by amplicon-size analyses of products from genes 24S and 18S, and the non-transcribed spacer of the mini-exon gene, and further characterized by sequence analysis of regions of the genes HSP-60 and histone H2A (H2A). Twenty-six samples were PCR-positive with primer set S35-S36, however only fourteen samples were successfully classified using genotyping gene-targets. Thirteen samples had T. cruzi lineage I, and one had a Trypanosoma spp. Sequencing of HSP-60 and H2A products was highly specific and results from 10 samples confirmed lineage classification. These findings suggest that more than one gene target is needed for proper identification and characterization of T. cruzi, and the predominant lineage circulating in La Joya prior to spraying appears to have been lineage I of T. cruzi.

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Geographic information system and remote sensing in the identification of risk factors involved in the urbanization of American Visceral Leishmaniasis in Feira de Santana, Bahia, Brazil.

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In Brazil, visceral leishmaniasis (AVL) is a chronic parasitic disease caused by infection with *Leishmania chagasi.* The transmission cycle may involve animals as reservoir hosts or man as the sole reservoir and sole source of infection for the vector. However, the domestic dog is the principal reservoir contributing to the maintenance and dispersion of the diseases. Migratory movements and modification of rural environments have facilitated the urbanization of AVL that now has been invading areas that used to be free of disease. To understand some factors that may be associated with AVL transmission in urban areas, we used geographic information system and remote sensing techniques in order to verify if the urban transmission to dogs and humans in this city is related to socioeconomic conditions, vegetation coverage, and climate features of the environment. Human and canine leishmaniasis cases reported from the state secretary of surveillance and health from 2000 to 2003 were geo-referenced and plotted on a map of the municipality (scale 1:2,000). Transformed normalized vegetation indexes (TNDVI) were extracted using a composition of LANDSAT 7 ETM+ satellite image. The area of study is composed of an area of 155 km^2 with 419,829 inhabitants and 41,983 canines distributed among 43 neighborhoods. The average age of individuals with VL was 13 years old and 64.8% of cases occurred among women. Significant correlation between human prevalence and TNDVI ($p=0.034$) and between canine incidence and TNDVI ($p=0.039$) was observed. A linear regression model using human prevalence, TNDVI, and temperature suggested that those variables may be useful for the study of AVL in an urban environment ($p=0.031$). It was also observed that as the family income decreased the number of positive dogs in the area increased ($p=0.001$) thus supporting the relationship between disease and poverty. GIS and RS can be a helpful source bringing valuable information for the understanding of the urbanization process of AVL in the municipality studied.

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Wide Distribution of Genetic Polymorphisms Associated with Sulfadoxine-Pyrimethamine Resistance among Plasmodium vivax isolates in Indonesia

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Wide Distribution of Genetic Polymorphisms Associated with Sulfadoxine-Pyrimethamine Resistance among *Plasmodium vivax* isolates in Indonesia
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Treatment failures associated with chloroquine within three decades in Indonesia has made sulphadoxine-pyrimethamine (SP) combination more widely used. Molecular basis of parasite resistance to SP has been established in *P. falciparum* and rodent plasmodia, and various single nucleotide polymorphisms (SNPs) in dihydropteroate synthase (*dhps*) and dihydrofolate reductase (*dhfr*) have been linked to the resistance. The SP combination has never been used to treat vivax malaria, however, the sympatric existance

of *P. falciparum* and *P. vivax* in malaria endemic regions of Indonesia and the practice of malaria treatment without microscopic confirmation suggest that the accidental treatment of vivax malaria with SP has often taken place. In an attempt to evaluate the use of SP for vivax malaria and for Intermittent presumptive treatment in pregnancy (IPTp), we have screened genetic polymorphisms in *dhps* and *dhfr* genes among the *P. vivax* isolates from several malaria endemic regions in Indonesia. The results revealed five SNPs in *Pvdhfr* gene: 13L, 57L/I, 58R, 61M, and 117N/T. The *Pvdhfr* 117Asn appeared either as single mutation or paired with 58-Arg in the frequency of 20-90% among the isolates examined. The triple (58R/61M/117T) and quadruple (57L/58R/61M/117T) mutations were found from in several isolates observed. Genetic polymorphism in *Pvdhps* gene occurred in much less frequency, and all found as 383G. The findings reveal distribution of SNPs in *Pvdhfr* gene among the *P. vivax* isolates in Indonesia but still indicate that SP may still be effective to be used either as monotherapy or in combination with artemisinin derivatives to treat vivax malaria.

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Spatial and environmental patterns of *Mycobacterium ulcerans* presence in southern Ghana

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Mycobacterium ulcerans infection (Buruli ulcer) is a skin disease that can result in devastating morbidity. It is endemic in many countries in central and western Africa. In Ghana, the distribution of BU is limited to the southern portion of the country, and the most endemic district has rate of 150.8 per 100,000 people. The mode of transmission remains elusive, although studies have found *M. ulcerans* presence in communities to be associated with flood water exposure. Here, we examine the spatial patterns of the environmental *M. ulcerans* presence. Aquatic environments were sampled and subsequently tested for *M.ulcerans*. Spatial statistics were applied to test for clustering of presence/absence of *M. ulcerans* in aquatic environments. A global case-control K-function was used to test for general trends of clustering, while a local Kulldorff's spatial scan using a Bernoulli model was applied to test for local clusters of *M. ulcerans* presence. A variety of satellite imagery were used to characterize the landscape. MODIS (Moderate Resolution Imaging Spectroradiometer) satellite imagery was used to determine landcover types and the extent of flooding areas around sites are annually. SRTM (Shuttle Radar Topography Mission) imagery was used to determine elevation and create hydrological models. Regression analyses were applied to examine how local site (e.g., flow and quality) and landscape (landcover, hydrology, elevation) variables may be related to the presence of *M. ulcerans*. *Mycobacterium ulcerans* presence was randomly distributed at both the global and local level. Regression analyses found that different local site characteristics varied between aquatic environments, and were associated with *M. ulcerans* presence. The different analytic methods reveal different components of the underlying spatial and environmental processes driving the distribution of *M. ulcerans*, and raise important hypotheses for future investigations.

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Plasmodium falciparum gametocyte carriage is associated with subsequent Plasmodium vivax relapse after treatment

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Up to one-third of patients in Southeast Asia develop P. vivax relapse shortly after treatment of what appears to be a P. falciparum mono-infection. In a 2006 drug efficacy trial in western Cambodia, 111 patients with uncomplicated P . falciparum malaria were randomized to receive either artesunate monotherapy or quinine plus tetracycline over 7 days. They remained hospitalized for 21 days of the 28-day follow up period. 32/107 (30%) patients developed P. vivax infection within 28 days, suggesting relapse from a cryptic vivax infection (CVI) present at admission. Only 3 of these (9%) had PCR-detectable P. vivax parasites in the peripheral blood from admission, suggesting that in the majority of patients who relapsed, P. vivax parasites resided in the liver at the time of presentation. When microscopic data of the 32 CVI patients were retrospectively analyzed, it was discovered that those with P. falciparum gametocytes on admission were more likely to develop relapse with P. vivax upon follow up (RR=2.4, 95% CI 1.4-4.1, p=0.003). In total, 24/107 (22%) patients carried smear-detectable falciparum gametocytes at admission and over half of them (13/24, 54%) relapsed with P. vivax within 28 days compared to 19/83 (23%) of non-gametocytemic patients who relapsed. Neither reported duration of illness prior to presentation nor initial parasitemia could explain the difference between groups. This association between falciparum gametocytes and cryptic vivax infection that later relapses suggests possible interspecies interactions between malaria parasites. The presence of a second competing malaria species may boost falciparum gametocytogenesis, leading patients who harbor mixed infection to contribute disproportionately to ongoing malaria transmission. Moreover, gametocytes seen at presentation may be a potential marker for liver-stage P. vivax infection.

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Mapping malaria risk in Bangladesh using Bayesian geostatistical models

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Background
Malaria control programs are dependant on accurate risk maps to effectively guide the allocation of interventions and resources. Detail of malaria risk in Bangladesh is sparing and this study aims to represent the first malaria risk maps for the country.

Methods
A comprehensive malaria prevalence survey (N=9750 individuals, N=309 communities) was carried out in 2007 across the thirteen known endemic districts of Bangladesh. Bayesian geostatistical models with environmental covariates were constructed and used to predict continuous *P. falciparum* prevalence across the extent of the malarious areas of Bangladesh. To explore different drivers of malaria transmission, the analysis was stratified by ecological zone.
Results
The average prevalence across the 13 districts was approximately 4% with the majority of cases *P. falciparum* (90%). Malaria in Bangladesh clearly exists in two differing ecological zones - a hilly forested area and a floodplain area. In the hilly forested area all available environmental covariates (precipitation, temperature, elevation, forest cover and access to major centre of greater than 500,000) were included in the final model. In the floodplain area only precipitation, elevation and access were incorporated to give the best predictive ability for the zone.
Conclusion
The risk maps reveal that malaria transmission is highly varied across the endemic districts and the maps would be an invaluable resource to the national malaria control program. Of particular concern is the highly endemic region bordering Burma which is known for its high levels of drug-resistant *P. falciparum*. This analysis also highlights the importance of stratification of analyses by ecological zone, even with a comparatively small nation such as Bangladesh.

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Disruption of the multidrug resistance associated protein (*pbmrp*) gene in *Plasmodium berghei*

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The development of widespread multidrug resistance *Plasmodium* has contributed to the increase in morbidity and mortality caused by malaria. Understanding the mechanisms responsible for the development of antimalarial resistance is necessary in order to develop alternate strategies to control and treat this disease. Overexpression of members of the ATP-binding cassette (ABC) transporter superfamily, one of the largest evolutionarily conserved families of proteins, have been found to contribute to drug resistance in organisms ranging from bacteria to man. These proteins play key roles in cellular detoxification of endobiotics and xenobiotics. Overexpression and single nucleotide polymorphisms within a homologue of this gene in *P. falciparum* (pfMRP1) have been associated with antimalarial resistance. In addition, disruption of pfMRP1 affects drug sensitivity profiles to several antimalarials. To elucidate the biological role of MRP and to examine its putative association to drug resistance, we have generated a *P. berghei* mutant line in which *pbmrp* was disrupted. *In vivo* drug sensitivity analyses show no difference between the wild type and the *pbmrp* ko response to chloroquine or artemisinin. Interestingly, the lack of this gene shows no fitness cost in *pbmrp* ko mutants when compared to wildtype in blood stages. Studies are underway to investigate the *pbmrp* ko phenotype during mosquito and liver stage development. This *pbmrp* ko line is an invaluable tool to investigate the biological role of *pbmrp* in addition to its putative role in *Plasmodium* drug resistance. Moreover, this line may facilitate the examination of the role of the *Plasmodium berghei* MRP in *Plasmodium* detoxification pathways and membrane transport mechanisms which may lead to the identification of novel drug targets or multidrug treatment strategies to combat malaria.

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<u>Globalization</u> and health in Shipibo-Konibo Communities in Ucayali, Peru: PHOTOVOICE experience.

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Introduction
In Peru there is an increase in the concentration of indigenous population in urban settings like Pucallpa, probably due to the process of the globalization. The main challenge in public health is to articulate a broader definition of health where interdisciplinary work, social determinants of poverty and inequality can be included. Photovoice, is a qualitative strategy that employs photography to assess health status.

Material and Methods:
Photovoice, a qualitative strategy was carried out in 3 shipibo-konibo communities (Nueva Era, San Francisco and Santa Teresita) located near the main city of Pucallpa in Ucayali, jungle of Peru. 10 adults and 2 children in each community were invited to participate. They were all given disposable cameras and asked to take pictures concerning what they believed was a problem or a goal in maintaining health inside their communities. All images were discussed in focal groups.
Results:
All 3 communities referred having problems in elimination of garbage, latrines, and dog's vaccination. In regards individual concerns; 1)San Francisco: Tourists and entrance of STD; 2) Santa Teresita's health post had no personnel to attend febrile pregnant women; 3) Nueva era was concerned of child malnutrition. 3 women in Santa Teresita asked for formal training as health promoters. Local photographic exhibition was done.
Discussion
The impact of photographs, coupled with personal stories, has certainly provided powerful insights in the Shipibo-Konibo people and has help them to point out their main concerns and problems regarding their perceptions of health. This strategy can help generate specific recommendations to incorporate indigenous perspectives into conventional health care and increase the relation between health care services and the community.

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Unit Based Surveillance (UBS) - The Royal Thai Army Early Warning System for Medical Threats along Thai-Northern Cambodia and Northern Thai-Myanmar Borders; Phase III

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An early warning system for medical threats in military areas of operation (AOs) along Thai borders has been developed to monitor diseases of military importance in near real-time in order to rapidly respond to disease outbreaks. The system was designed as a military unit-based surveillance where medical information is collected, entered into a database and electronically sent to AFRIMS for analysis. Information on any potential outbreaks will be immediately reported to the Ministry of Public Health (MOPH) and the Royal Thai Army Medical Department for outbreak response. The system, called Unit Based Surveillance (UBS), has been implemented in AOs along the Thai-northern Cambodia border since 2002 and along the Thai-southeastern Myanmar border since 2004. Over the past 7 years, we have obtained medical information in these AOs where the MOPH was unable to perform routine surveillance, and we also learned several lessons from both successes and failures which led to the third phase of this project. In this phase, we made several enhancements including improving data transmission capabilities and development of a new version to incorporate simplified symptom-based syndromes compatible with military readiness needs and the MOPH national surveillance system. The data collection and submission process has been designed to practically operate in 13 surveillance sites covering AOs in Thai-north Cambodia and northern Thai-Myanmar border areas. Since June 2009, febrile diseases, malaria and respiratory illnesses accounted for 75% of illnesses reported in Thai-north Cambodia AO, and also accounted for the majority of diseases along with musculoskeletal illnesses in the northern Thai-Myanmar border area. With the onset of new disease outbreaks, such as the novel H1N1, we can monitor remote areas that normally have no disease surveillance or in-depth medical care and can assist in tracking transmission of new diseases across borders. In conclusion, a practical early warning system along Thai borders was developed and tested. The ultimate goal is to fully implement the system to enhance the national disease surveillance system.

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Evaluation of a New Immunochromatographic Test using Recombinant Antigen B8/1 for the Diagnosis of Cystic Echinococcosis

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Human cystic echinococcosis (CE) is a parasitic disease caused by the cestode *Echinococcus granulosus*. This zoonosis mainly affects population dedicated to livestock rising in developing countries, with clinical symptoms usually appearing several years after infection. Immunological tests are important methods to confirm clinical diagnosis in areas where imaging techniques are not accessible. Conversely to other available assays, immunochromatographic test (ICT) offers immediate, bed side diagnosis. We evaluated an ICT for the rapid detection of antibodies to *Echinococcus granulosus* using two recombinant antigens, AgB8/1 for CE and Em18 for AE (Adtec Co. Ltd., Oita, Japan). We analyzed sera samples from 50 confirmed CE cases which included individuals with lung or liver cysts, including cases both with and without complications. The overall sensitivity of the ICT was 78% (39/50). Sensitivity of the assay for lung complicated and non-complicated cysts was 83.33% (10/12) and 69.23% (9/13), respectively, whereas

in liver complicated and non-complicated cysts it was 83.33% (10/12) and 76.92% (10/13). Qualitative intensity assessment in non-complicated cases showed much weaker reactions in lung cysts compared to liver, suggesting better evasion mechanisms of the parasite in the lungs. Additionally, we selected subsets of CE and neurocysticercosis samples to be processed with an Alveolar Echinococcosis ICT assay. Only one sample of complicated pulmonary hydatid case cross-reacted

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Disruption of *Plasmodium berghei* glutathione reductase compromised parasite development in the sexual stages

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The complex life cycle of *Plasmodium* spp., etiological agent of malaria, renders the parasite with high levels of oxidative stress. To cope with these elevated levels of oxidizing agents the parasite depend on their thioredoxin and glutathione (GSH) antioxidant systems. The potential role of the glutathione redox system in *Plasmodium* drug resistance has been widely studied. GSH levels have been shown to be higher in chloroquine (CLQ) resistant lines as compared to the sensitive lines. Additionally, several enzymes involved in GSH metabolism have been proposed as antimalarial targets. *Plasmodium* parasites have a very efficient GSH homeostasis system which includes *de novo* GSH synthesis and recycling. Glutathione reductase (GR), the major reductant of glutathione disulfide (GSSG) in *Plasmodium,* has also been proposed as target. The main objective of this work was to evaluate the role of GR in *P. berghei* development along their life cycle by means of gene disruption. Correct integration of the disruption construct was confirmed by Southern and chromosome analysis. Furthermore, no pbGR activity was detected in parasite extracts from *P. berghei* GR deficient parasites (*pbgr*$^-$). Development of *pbgr*$^-$ parasites was normal during mice infection. No statistical difference was found between the GSH levels of *pbgr*$^-$ and wild type parasites. In addition, there were no differences between sensitivity levels to CLQ or artemisinin in *pbgr*$^-$ and wild type parasites. Analysis of *pbgr*$^-$ mutants during the sexual stages shows impaired oocyst development resulting in smaller oocysts than wild type in *Anopheles stephensi* mosquitoes. Moreover, transmission of *pbgr*$^-$ mutants to naïve mice was blocked. Our results question the suitability of pbGR as a potent drug target and also the involvement of GSH in the development of drug resistance.

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Effectiveness of commercially available long-lasting insecticide treated nets on fifth instar *Rhodnius prolixus*, *Panstrongylus megistus*, and *Triatoma dimidiata* from colony

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Long-lasting insecticide treated nets (LLIN) are commercially available with different types of insecticide and are commonly used to protect individuals from disease carrying mosquitoes. These LLINs are useful tool against other disease carrying insects including triatomine bugs. Some species of triatomine bugs serve as vectors of Chagas disease in Central and South America as well as parts of southern Texas, and each species responds differently to different insecticides. Our objective was to determine the effects of three commercially available LLINs; PermaNet, Duranet, and Olyset Net, on three species of triatomine bugs; *Rhodnius prolixus*, *Panstrongylus megistus*, and *Triatoma dimidiata*, for five, 10, 30, and 60 minute periods. Colony reared bugs were used seven days after molting to the fifth instar, and World Health Organization (WHO) cones were used to expose the bugs to the netting. Twenty fifth instar nymphs were used for each test group, totaling 320 individuals of each species used for each replicate, and a total of three replicates were completed. Of the three nets tested, the Duranet is most effective against all three species of triatomines in terms of total number of bugs knocked down as well as shortest time until knock down at 10 minutes. These findings are important because knowing the LLIN that is most effective against triatomines will help to reduce their populations as well as Chagas transmission. Using Duranets in addition to the more traditional control method of residual spraying would help to target domestic as well as sylvatic populations of triatomines. The Duranet would also be effective against triatomines for up to five years and through 30 or more washings, unlike residual sprays which require repeat application within a year.

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Cross-Sectional Study of Household Risk Factors Associated With Human Cystic Echinococcosis In An Endemic Region Of Peru

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Cystic echinococcosis (CE), a zoonosis caused by the larval stage of the tapeworm *Echinococcus granulosus,* has a worldwide distribution and has often debilitating effects on infected individuals with severe economic repercussions on their communities. In developing countries, the definition of high risk sub-groups would optimize the use of resources and improve the effectiveness of screening programs. To this aim, we performed a cross -sectional study using information about 417 houses from a community based screening performed in Junin which is considered a Peruvian endemic area. Specifically, we determined household characteristics that could be associated with at least one positive case among their members. Fifty six (13%, 95% CI: 10.6 %-16.7%) households had at least one CE case diagnosed either by abdominal ultrasound or chest X-ray among their members. After controlling for household characteristics and evaluation coverage, household with more than 3 members (OR=2.44; 95% CI: 1.07 - 5.58), located in the lower poverty quintile (OR=2.51; 95% CI: 1.12 - 5.61), and those which reported raising animals in the house (OR=2.35; 95% CI: 1.10 - 5.03), were statistically more likely to have at least one CE case. Household characteristics described above are related to a high level of poverty, and in our analysis are the best-defined risk factors for human infection with CE. The association between zoonoses and poverty has been gaining attention in recent years, and poverty alleviation is currently one of the WHO-FAO proposed approaches to target zoonoses control.

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Socio-ecological risk factors for dengue fever transmission in Guayaquil, Ecuador

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Emerging infectious diseases pose a heavy social and economic burden on populations in developing countries. Dengue fever (DF), a virus transmitted to humans primarily by the Aedes aegypti mosquito, is the most significant and rapidly spreading vector-borne virus globally. With no vaccine currently available, an estimated 2.5 billion people in over 100 countries are at risk for DF. The objective of the study was to identify socioeconomic factors that increase the risk of DF transmission in Ecuador. In June 2008, we launched a pilot study of dry season DF transmission in Guayaquil, Ecuador, a coastal city with over two million people. We conducted over 300 household surveys in two low-income communities with different historical incidences of DF. At each household, we collected all visible mosquito pupae and used questionnaires to determine the (1) history of DF infection, (2) at-risk behavior, and (3) household characteristics that affect transmission (e.g., screens on windows). The results of this preliminary analysis indicate that there are significant socio-ecological differences between the neighborhoods, such as water storage practices and vegetation cover, that may contribute to the higher historical incidence dengue in one neighborhood. This critical research will aid in the development of a dynamic, spatial model of disease transmission that will aid public health policy makers to mitigate the burden of DF.

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Global Distribution of Outbreaks of Water Related Infectious Diseases and Risk Factors

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Water plays a vital role in the transmission of many infectious diseases and these water related diseases pose a great burden on global public health. In this study we describe global distribution of water related infectious diseases and explore potential risk factors associated with spatial distribution of outbreak events. Outbreak events from 1991 to 2008 were collected from the Global Infectious Disease & Epidemiology Network (GIDEON). Also collected are socio-economical and environmental factors including global population density (2000), annual accumulated temperature, and per capita water use. To adjust for potential reporting bias in outbreak reporting, publication frequency in bio-medical journals from each country is used as a covariate. A Bayesian spatial model is used to explore the impact of potential risk factors on the distribution of these outbreak events and global relative risk maps based on the model predictions are generated.
A total of 2 092 outbreak events are included in the study, among which 80.1% (1676) are associated with water/food-borne diseases, and 41.5% (869) associated with emerging or reemerging diseases. 56.5% (1182) outbreak events are caused by bacteria, 34.9% (730) by viruses, and the rest by parasites. Results show that the outbreak events are significantly correlated with socio-economic and environmental factors. Population density is a risk factor for all categories of water related diseases; annual accumulated temperature is a risk factor for water-washed and water-related diseases; and per capita water use is reversely related to outbreak events associated with water/food-borne and water-related diseases. The models predictions suggest that west Europe, central Africa, north India, Japan are the high risk areas for water/food-borne diseases (e.g. *Escherichia coli* diarrhea); west Europe, north Africa, and Latin America are more vulnerable to water-washed diseases (e.g., conjunctivitis), and water-based diseases (e.g., schistosomiasis) are more likely to occur in east Brazil, north-west African and south-east of China; and high risk areas for water-related diseases (e.g., Dengue fever) are focused in central Africa and India.

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Changes in immunoglobulin G levels to P. falciparum antigens in areas of stable as compared to unstable transmission

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Introduction:
Adults in areas of unstable transmission remain susceptible to clinical malaria, while adults in areas of stable transmission are generally protected against clinical malaria. Immunoglobulin G (IgG) antibodies to specific P. falciparum antigens have been associated with protection from clinical malaria. The persistence of these antibodies over time in areas of unstable as compared to stable transmission has not been well characterized.
Methods:
Levels of IgG antibodies to six P. falciparum antigens (CSP, LSA-1, TRAP, AMA-1, EBA-175 and MSP-1) were measured in 106 individuals in an area of stable transmission (age range, 6 months to 70 years) and 90 individuals in an area of unstable transmission (age range, 10 months to 75 years). Levels were measured at the same times in both sites, first after the short rainy season (November, 2000) and later after the long rainy season (August, 2001).
Results:
In older children (>15 years of age) and adults, IgG levels to CSP and MSP-1 decreased over time in the unstable transmission area but were unchanged in the stable transmission area. In contrast, IgG levels to TRAP, AMA-1 and EBA-175 decreased over time in both areas, and IgG levels to LSA-1 were unchanged in both areas. In children <15 years of age, IgG levels to all antigens except LSA-1 decreased over time in the area of unstable transmission, while only IgG levels to MSP-1 and TRAP decreased in the area of stable transmission.
Conclusions:
Changes in IgG levels to P. falciparum antigens vary according to local transmission intensity, age and antigen. The increased susceptibility of older children and adults to clinical malaria in areas of unstable as compared to stable transmission may be related in part to the decrease in IgG levels to CSP and MSP-1 seen in these individuals in the absence of repeated exposure.

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Determinants for the formation of the LRIM1/APL1C complex, a component of the mosquito complement-like pathway involved in defense against Plasmodium parasites

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The *Anopheles gambiae* leucine-rich repeat proteins LRIM1 and APL1C circulate in the hemolymph as a disulfide-bonded complex. This complex is a crucial component of the mosquito complement-like pathway that is important for immune defense against *Plasmodium* parasites. The LRIM1/APL1C complex interacts with the C3-like protein, TEP1. This interaction stabilizes the processed form of TEP1 and is required for its localization to the parasite surface. Interestingly, complex formation is required for the secretion of these proteins as neither the complex nor monomeric LRIM1 or APL1C are detectable in the hemolymph if either gene is silenced by RNAi. In contrast, cultured mosquito cells will secrete homomeric complexes and monomers of LRIM1 and APL1C into conditioned medium. Similar to hemolymph, robust secretion is only achieved when both proteins are produced, and in this case, the LRIM1/APL1C heteromeric complex is the preferred form. Given that LRIM1 and APL1C belong to a protein family of putative innate receptors with shared structural features (named LRIMs), we are interested in the specificity of complex formation, how the complex functions in immune reactions and what regions of the proteins are required for interactions with TEP1 and other pathway components. To understand these questions, we have generated a set of LRIM1 and APL1C structural variants and expressed them in cell culture. Preliminary data indicate that LRIM1 and APL1C have an intrinsic ability to form a complex and that its assembly occurs in a specific order. Our finding that discrete modules control how these two proteins interact during synthesis leads to the hypothesis that evolutionary exchange of these could result in novel LRIM combinations thereby generating unique pathogen specificity.

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Serodiagnosis of human cystic echinococcosis: Evaluation of the B2t-ELISA tool in Peruvian patients

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Diagnosis of cystic echinococcosis (CE) is mainly based on the use of radiological techniques, limiting the use of serological techniques as a complementary tool to follow up patients and to confirm presumptive cases. In previous studies, the use of recombinant antigens derived from Antigen B, a parasite molecule, demonstrated different levels of success. In our study a recombinant antigen derived from the subunit AgB2 of AgB were tested with enzyme-linked immunosorbent assays (ELISA). A cross sectional study using serum samples from 136 surgically confirmed cystic echinococcosis (CE) patients and 60 patients with a presumptive clinical and/or radiological diagnosis of CE was performed in terms of their radiologic characteristics, serologic response, cyst characteristics and organ involvement. The majority of our cases came from the thoracic surgery departments of major referential hospitals. Cysts were mostly single and located in lung. Liver compromise was found in 35% of the patients, and compromise of more than one organ was found in 17% of the patients. Twenty five patients had previous cystic echinococcosis. Serology using the recombinant AgB2t in ELISA for the detection of total IgG was 78.97% sensitive. Seropositivity was not statistically associated with organ involved or CE WHO-cyst type classification, but it was clearly associated to the number or cysts and to the previous treatment of the patients.

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Pharmacokinetics and Pharmacodynamics of 7 day oral artesunate monotherapy in volunteers with uncomplicated P. falciparum malaria in Western Cambodia.

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Antimalarial drug resistance, particularly to artemsinins along the border of Cambodia and Thailand is an important public health concern raised in recent years. The pharmacokinetics and pharmacodynamics of oral artesunate monotherapy were explored as part of a multicenter surveillance study to contain antimalarial drug resistance. Patients with uncomplicated P. falciparum malaria at Tasahn Health Center in Western Cambodia were enrolled in an open label study of 7 day directly observed oral artesunate monotherapy at 3 dose levels (2,4 and 6mg/kg). There were not significant differences in clinical outcomes between the 3 dose groups, but wide variability in artesunate concentrations were observed despite weight-based dosing. There were significant reductions in plasma concentrations between day 1 and day 7 of dosing, suggesting autoinduction of metabolic clearance pathways. Parasite clearance times were prolonged compared to previous studies, suggesting early stage clinical resistance. Dose limiting hematologic toxicity with neutropenia in 5 of 26 subjects occurred at the 6mg/kg dose level. Data to support alternative therapeutic strategies will be discussed.

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Temporal and spatial distribution of seasonal and pandemic influenza in Mexico

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Temporal and spatial distribution of seasonal and pandemic influenza in Mexico
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The long-term purpose of this project is to develop a system that can predict the timing and impact of seasonal and pandemic influenza. The analysis of historical and recent data indicates that in Mexico, seasonal and pandemic influenza occurs with a repetitive pattern. Trends of synchronized peaks with equivalent morbidity and mortality rates allowed us to identify five “influenza provinces”, each with a similar pattern of seasonal and pandemic influenza. Each “province” clusters neighboring states. Notably and consistently, one of these clusters displays the highest rates of influenza in Mexico. The states that are included in this cluster form an “influenza corridor” in the central part of the Country, along the valleys running between the oriental and occidental sierras and extending from the Valley of Oaxaca to the state of San Luis Potosí. The epidemiological trends are independent of population density and are influenced by climate, particularly minimum temperature and absolute humidity. The results suggest that some degree o predictability may be achieved in the future.

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Evaluation of an ELISA Kit for cystic echinococcosis using a lipoprotein fraction from fertile bovine fluid cyst

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Cystic echinococcosis (cystic hydatid disease, CHD) is one of the most prevalent human cestode infections in humans. It can develop in almost any part of the body, with different radiological findings and complications. Image is the diagnostic method of choice, with serology as a helpful tool for confirmation of suspected cases. The performance of available serological assays for hydatid disease is sub-optimal and more information is needed on their advantages and limitations.
We used an ELISA Kit (CELQUEST - Celsius S.A., Montevideo, Uruguay) based on a lipoprotein fraction from fertile bovine cyst fluid of *E. granulosus* and purified by liquid chromatography using an acrylic heparin column. This assay was evaluated using 368 anonymized archive sera samples with ethic approval for subsequent use. Samples included 177 from surgically confirmed cystic echinococcosis, 101 negative controls from residents from a non endemic area, without any suspected of disease, and 90 sera from heterologous infections: 20 from neurocysticercosis (NCC) by *T. solium*, and 70 from individuals harboring a single helminthic infection: *H. nana* (n=23), *A. lumbricoides* (n=15), hookworm (n=8), *E. vermicularis* (n=8), *S. stercoralis* (n=8) y *T. trichiura* (n=8). The sensitivity of the assay was 85.3%, with a specificity of 96%. No differences were found in relation to the location or number of lesions. Cases with complicated, ruptured or infected cysts were more frequently seropositive than those without complications (91.1% versus 75.4%, p=0.004). Cross reactions were found in cases with NCC (60%) and hookworm (37.5%). This assay provides a suitable alternative for the serological confirmation of CHD.

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Potential Therapeutic Use of Transforming Growth Factor-β for Treatment of Hantavirus Cardiopulmonary Syndrome

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Sin Nombre virus (SNV) was first identified in 1993 in the Four Corners region of North America as an etiologic agent of hantavirus cardiopulmonary syndrome (HCPS). Infection is associated with high levels of inflammatory cytokine staining in human pulmonary autopsy specimens, suggesting HCPS is an immunopathology. The reservoir of SNV is the deer mouse (Peromyscus maniculatus), which develops persistent infection without pathology. Experimental data suggest increased expression of transforming growth factor beta-1 (TGFβ1) in these animals. The Syrian golden hamster (Mesocricetus auratus) has been used as an HCPS model with Maporal virus (MAPV). We are examining the potential of TGFβ1 as a therapeutic agent in this model. We administered 200 ng of TGFβ1 using osmotic pumps to examine its effects on the inflammatory response to MAPV. Hamsters infected with MAPV and treated with TGFβ1 had significantly decreased lung congestion and pleural fluid compared to untreated infected hamsters, although the death rate was not reduced. Gene expression of IFNγ was increased approximately 15 fold over control in lung tissue while IFNγ, IL-1β and IL-4 were also increased 4 fold over control samples from spleens. We are currently processing tissue samples from these hamsters to examine histopathology and viral load, and are planning to repeat the experiment using a higher dose of TGFβ1 to determine if it may be more effective at higher doses.

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Cellular Analysis of the Immune Response in Active and Scarring Trachoma Using Flow Cytometry

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Active trachoma and trachomatous scarring are immunopathological diseases caused by C. trachomatis. Although recent work has shown variation in the expression of multiple cytokines and fibrogenic factors in both active and scarring trachoma cases, there has been no analysis of these changes at a cellular level. The purpose of this study was to examine changes in cell number, type and expression of mucins and key immunomodulatory molecules in both active and scarring cases of trachoma. Clinical signs of trachoma were assessed using the WHO simplified grading scale; 36 cases of active trachoma and 17 cases of scarring trachoma (non active; TS) were identified in the Kombos and Kiang Districts in The Gambia. Cells from both cases and paired controls were obtained from the conjunctivial tarsal with a swab and analyzed using polychromatic flow cytometry. In active trachoma cases there a significant increase in both total number of cells (p=.04; 32,951 v. 21,659) and lymphocytes (CD45+; p=.017). There was no significant difference in either total number of cells or lymphocytes for TS cases, suggesting a specific and short-lived cellular response. In active trachoma cases, significant differences were observed in both the overall number and proportion of T lymphocytes (CD45+ CD3+; p<.01) as well as helper (CD45+ CD3+ CD4+; p<.01) and cytotoxic T cell (CD45+ CD3+ CD8+; p=0.37) counts, further suggesting a role for T cells in the trachoma immune response . There appears to be a marginally significant increase in monocytes (CD 45+ CD14+) and B lymphocytes (CD45+ CD19+). There are no significant differences in any of these cell types in TS cases. This study, the first to analyze trachoma cases with flow cytometry, provides further insight into the cellular basis for the trachoma immune response. Comparison with C.trachomatis load as well expression levels of cytokine and fibrogenic factors will enable a fuller understanding of the immune response to trachoma.

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Multiplicity of Plasmodium falciparum clones in the process of adaptation to in vitro culture of infections from malaria endemic areas of the Peruvian Amazon

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Introduction
Plasmodium falciparum is a highly polymorphic parasite, which allows it to evade the host immune response, spread drug resistance and enhance disease transmission. It is now well-established that strains of P. falciparum found in regions of high transmission are genetically diverse, and that humans as well as mosquitoes are often infected by multiple parasite clones. In this study, committed to understanding the genetic complexity of the P. falciparum parasite, analysis was performed to evaluate the genetic diversity of P. falciparum strains in the process of adaptation to in vitro culture during the years 2005 to 2008 in the low transmission Peruvian Amazon.
Methods: Samples from P. falciparum infections during the years 2005-2008 were collected from malaria endemic communities near Iquitos, Peru. These were cultured in vitro for up to two months. Of 130 isolates that grew successfully, DNA was purified at different culture time points (0 to 45 days) and analyzed by semi-nested multiplex PCR to confirm the presence of the parasite. The genotyping was done by nested PCR of Block 2 of the msp1gene.

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Is twice daily dose of artesunate is better than single dose in treatment of severe falciparum malaria?

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Severe malaria remains a major cause of mortality in the world. Although there is increasing evidence that parenteral artemisinin derivatives may reduce mortality but it is still high. Improved means of treating severe malaria are urgently needed. We examined the two regimes of parenteral artesunate to assess the safety, tolerability and effectiveness of treatments for strictly defined severe falciparum malaria. 200 patients admitted with severe falciparum malaria in Bangkok Hospital for Tropical Diseases were recruited into this study. Patients were assigned to one of two treatment groups: (i) 5 days course of intravenous artesunate twice daily (1.2 mg/kg q 12 h) followed by mefloquine (25 mg/kg) and (ii) 7 days course of intravenous artesunate once daily (2.4 mg/kg q 24 h). When patients could take oral medications, the parenteral antimalarials were administered as oral agents. No neurological sequelae were observed. The mortality was 1% and 3%, respectively. In addition, the parasite clearance (54 h vs 57 h) and the fever clearance times (69 h vs 87 h) were slightly shorter in group (i). However, the cure rate (92% vs 73%) was significantly higher in group (i). The treatments of both regimens were safe and well tolerate but a combination of parenteral artesunate twice daily followed by mefloquine was highly effective in both morbidity and mortality.

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A molecular assay provides a novel capability for the rapid and comprehensive detection of multiple genomic segments of viruses of the family *Bunyaviridae*

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A molecular assay provides a novel capability for the rapid and comprehensive detection of multiple genomic segments of viruses of the family *Bunyaviridae*
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We present an RT-PCR based assay for the rapid detection and identification of all three genomic segments (L, M, and S) of viruses of disparate classification within the family *Bunyaviridae*. Using a limited set of universal primers, this assay amplifies cDNA from each bunyaviral genomic segment, followed by nucleotide sequencing of the PCR amplicons and NCBI BLAST analyses for speciation of the cDNAs. The analytical sensitivity and specificity of this assay were determined through the evaluation of RNAs extracted from selected bunyaviruses and other representative arthropod-borne viruses of diverse origins. In the diagnostic setting, this assay has been

used to quickly and comprehensively characterize samples of public health import; including a recently derived isolate of La Crosse virus from a pool of *Aedes albopictus* that was collected in Dallas county Texas, August 2009. The successful application of this assay, which provided genotype level identification of the Texas La Crosse isolate, suggests its usefulness for the identification of emergent bunyaviruses. The rapid detection of multiple segments of the bunyavirus genome provides an optimal capability for the identification of reassortant viruses within the family *Bunyaviridae.*

3203

Phylogeny and associated bacteria of wild populations of the tsetse fly *Glossina fuscipes* studied using next generation deep sequencing: implications for vectorial capacity and control

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The tsetse fly *Glossina fuscipes* is the major vector of human African Trypanosomiasis. In 2004 more than 75% of the 17,000 reported cases of chronic sleeping sickness cases occurred in areas where *G. fuscipes* is thought to be the principal vector. Three allopatric *fuscipes* subspecies have been defined on the basis of morphology. We present the results of a macrogeographic survey across the range of this important vector, examining the molecular evidence for the three putative subspecies and factors related to their vectorial capacity. Transmission of the disease causing trypanosomes *Trypanosoma congolense* and *T. brucei* species relies on their survival and establishment in the fly midgut. Tsetse fly midguts all harbour a primary symbiont, *Wigglesworthia glossinidia*, and some contain a secondary symbiont, *Sodalis glossinidius*. The available evidence suggests that the presence, abundance, and strain of symbiotic bacteria residing in the midgut affect fly susceptibility to trypanosome establishment. We screened *fuscipes* populations for the presence of *Sodalis* by PCR, and did not detect *Sodalis* in Lake Victoria basin *G. fuscipes*. We investigated tsetse associated bacteria further by 454 (Roche) sequencing of a portion of the bacterial 16S gene in DNA extracted from whole flies. We obtained >10,000 reads from each population sample, allowing estimation of the relative frequencies of different bacterial species of the tsetse microbiota, and present data contrasting the bacteria associated with *fuscipes* populations compared to other tsetse species.

3205

Adapting field-isolated, mixed *Plasmodium falciparum* isolates from malaria endemic areas of the Peruvian Amazon to *in vitro* culture

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It is well-established that *Plasmodium falciparum* is capable of introducing extensive genetic diversity. Consequently, *P. falciparum* is a hardy parasite that has an increased capacity to adapt to new and changing environments. It has been shown, especially in high transmission regions, that humans and mosquitoes are both routinely infected with complex (mixed-clone) infections. In such regions, determining how complex infections grow and respond *in vivo* or *in vitro* is too complicated to ascribe to a particular group of different parasites in an infection. Here, in this hypoendemic region where complex infections are infrequent, we aimed to look at the genetic complexity of parasites in association with adaptation of complex *P. falciparum* infections to *in vitro* culture.
***P. falciparum* infections between years 2005 and 2008 were collected from 13 villages near Iquitos, Peru. Strains from these infections (N=130) were cultured *in vitro* for up to 2 months. DNA was extracted at culture time points (0-45 days) and amplified by PCR to confirm parasite presence. Genotypes were determined by amplifying MSP1-B2. Behavior was characterized by: change in allele or number and also if alleles alternate or remain in tandem over time. As a baseline, genotypes were determined at day 0 of collection.**
Each of the 3 known allelic families were detected and successfully raised in culture. Of the 130 infections cultured: 80 (61.5%) were single-clone infections and 50 (38.5%) were complex infections. *Single-clone isolates:* no change observed in 46 (57.5%) of the isolates, while new allele appeared in 29 (36.3%) isolates and 5 (6.3%) lost an allele. *Complex isolates:* no change observed in 36 (72%) of the isolates, while 14 (28%) lost an allele over time. Alleles were found to alternate and remain in tandem over time. We found a higher proportion of complex to single-clone infections survive to ≥Day 21 in culture, 44.0% (22/50) and 32.5% (26/80), respectively. Parasite density did appear to impact culture survival; however, this was irrespective of the number of clones in the culture.
Parasites from complex infections tend to adapt more successfully to *in vitro* culture compared to single-clone infections. This may be due to an ability to alternate between different allele, rather than exhaustive replication of the same allele over time, as in single-clone infections. Further studies are necessary for the studying of the synchronization of such complex infection.

3206

Occurrence of Taenia multiceps infection in Israel-A new Zoonosis

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Taenia multiceps (Coenurus cerebralis) infection is a common and worldwide problem of small ruminants with a worldwide distribution. Dogs harboring the adult worm play an important role in spreading the disease. Coenurosis may develop in the brain, spinal cord and in other tissues of a wide range of animals, including sheep, goats and some wild animals. In Israel the disease was first describe by Landau et al at 1956 herd in central Israel. Since then no new information regarding the prevalence of the disease in Israel was reported. During the period 2000 -2008 a prevalence of 1.3 to 9.8% was demonstrated by us in some herds in central and south Israel, leading to mortality (1.14-24.61%) and culling of animals to the extent of 37.4%. High variability was observed regarding the cyst locations and size. Most infections were demonstrated in 0.5-3 year's old sheep. Clinical syndromes include vivid types of nervous symptoms with little or no change in haematological and biochemical profile. Treatment of coenuruses in sheep and goats using albendazole, niclosamide and praziquintal is only partially effective. coenurosis is a rare disease in humans and less than 100 cases were reported from Africa, the United Kingdom, France and North America. Recently, in the Negev desert area in southern Israel, an unusual case of a huge intraparenchymal cyst in a 4-year-old girl caused by T. multiceps was demonstrated.

3207

Fluorescent In-Situ Hybridizations Assays (P-Genus and PFV- FISH) for Detection and Differentiation of Plasmodium Species Directly on Blood Smears

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Despite enormous and diverse efforts to control malaria, it remains amongst the three most deadly communicable diseases. Malaria can be a life-threatening disease, especially in children, if left untreated; between 525,000 to 2.0 million African children die every year. The current gold standard for diagnosis is examination of Giemsa stained smear by microscopy. However, when parasite levels are very low, or in mixed infections, the information obtained by examination of Giemsa stained smear by microscopy is limited. Thus we have developed (1) P-Genus FISH assay that detects all the species of malaria causing parasites on an air-dried blood smear; and (2) Dual probe PFV-FISH assay that detects and differentiates P. falciparum (PF) and P. vivax (PV) on a single slide. The assays are simple and in-expensive. The only requirement is a fluorescent microscope. The assays consist of six steps: pretreatment, fixation, hybridization, washing, counterstaining and viewing the processed smear under a fluorescent microscope. The total assay time is approximately 1 hour. The limit of detection is between 1 to 9 parasites per 300 fields at 1000X. In preliminary studies performed on over 300 patients, the sensitivity of the FISH assays as compared to PCR was 83-89%, whereas Giemsa sensitivity was between 54-60%. Two independent clinical studies are currently underway. To date, 100 blood smears representing 89 patients (one sample from 78 patients and two samples from 11 patients, one collected on the initial visit and the second sample collected a week after treatment) have been tested by the FISH assays. 60 of the 89 patients were positive. 23 patients were positive for PV, 17 were positive for PF and 20 were positive for both PV and PF. The remaining 29 were negative. By other methods (including QBC, Binax Now® Malaria Test[RDT] , or Giemsa), only one species of Plasmodium was detected in 7 of the 20 patients with mixed infections. Additionally, 8 of the 11 patients tested a week after treatment were still positive by FISH, whereas all were negative by other methods. Based on the FISH results, the parasitemia had dropped in 5 patients, whereas in 3 patients there was no change in the parasitemia. The sensitivity and specificity of the FISH assay as compared to Giemsa and RTD was 100% and 81% respectively. After discrepant analysis the specificity of the FISH assays was 100%. However, the sensitivity of Giemsa and RDT as compared to FISH assays dropped to 81%.

3208

Parasites Hijack Host Calpain to Egress from Host Cells

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Apicomplexan parasites are obligate intracellular pathogens, with a complex life cycle, wherein the asexual phase is comprised of a virulent lytic cycle in which parasites invade and establish an intracellular niche within the host cell creating a specialized compartment to generate multiple daughter parasites. The resulting daughter cells must then break through both this intracellular vacuolar membrane and the host cell plasma membrane to allow for the infectious cycle to continue. Parallel studies on *P. falciparum* and *T. gondii* have led us to the discovery that apicomplexan parasites hijack host calpain proteases to facilitate their escape. Combining cell biological, pharmacological and genetic approaches, we provide evidence that both *Plasmodium* and *Toxoplasma* hijack host cell calpain proteases to facilitate parasite egress. Immunodepletion or inhibition of calpain-1 in hypotonically lysed and

resealed erythrocytes prevents the escape of *P. falciparum* parasites, but egress can be restored by reconstitution with purified calpain-1. Similarly, the efficient egress of *T. gondii* from mammalian fibroblasts is blocked by either siRNA-mediated suppression or genetic deletion of calpain activity, and can be restored by genetic complementation. Calpain mediated egress thus appears to be a general feature of apicomplexan parasite biology, suggesting the concept of targeting common host pathways for chemotherapy for a range of parasites, a strategy that could also limit the emergence of drug-resistant parasites.

3209

Increased Detection of Respiratory-associated Pathogens in Patients with Fevers of Unknown Origin to Health Facilities in Western Province Kenya

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Introduction: Due to the unprecedented efforts in malaria control by the global communities, malaria is on the decline. Unfortunately, attendance to hospitals by children with fever has not matched this decline. The number of infectious etiologies of fever of unknown origin especially in children is vast. A broad-spectrum analysis for potential fever-associated pathogens, including respiratory tract potential agents was adopted. The latter were detected using a multiparameter RespiFinder assay, which is based on the multiplex ligation-dependent probe amplification (MLPA) technology, and able to differentially identify 15 respiratory viruses.
Methods: Nucleic acid was extracted from 150 nasal wash samples collected from patients with fevers other than those caused by malaria, presenting to 3 health facilities in Western Kenya. MLPA analysis was performed as earlier described (Reijans et al., 2008). Amplified MLPA products were detected by capillary electrophoresis using an ABI 3130 genetic analyzer.
Results: The RespiFinder assay detected the following viruses: rhinoviruses (6%), influenza A (24%), influenza B (30%), human metapneumovirus (5%), adenovirus (11%), parainfluenzas (2%), respiratory syncytial viruses (6%) and coronaviruses (8%). Most patients presented with single infections, however patients suffering both dual (12%) and triple (4%) respiratory-associated infections were also detected.
Conclusion: Acute febrile infections remain a common presentation to health care facilities in Kenya. Although patients are more often empirically treated for malaria without substantial laboratory evaluation of acute causes, these finding indicated that respiratory-associated pathogens continue to exact a huge toll, especially in the under 5 population. It therefore remains imperative that in malaria endemic regions, diagnosis should also include the detection of respiratory-associated pathogens to allow for proper guidance in disease treatment, prevention and control strategies.

3210

Diversity of inherited bacteria in European ticks and their interactions with tick-borne pathogens

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Ticks have been qualified as “epidemiological zoos”, as they serve as hosts to a wide diversity of distantly related pathogens. It is less known that ticks also carry a diversity of microorganisms that live in close association with their hosts and have a diverse array of effects on their partners, ranging from mutualistic to parasitic. Several of these bacterial endosymbionts, acquired by ticks by feeding on infected hosts or by vertical transmission through eggs, have been identified in several tick species of major medical and veterinary importance, but their incidence, prevalence and effects on the biology of their hosts and on the epidemiology of tick-borne diseases remain largely unknown. Here, we genetically characterized the presence of a diverse array of tick-borne pathogens and endosymbionts in three tick species of major medical and veterinary importance (*Ixodes ricinus* N=296, *Dermacentor reticulates* N=86 and *Rhipicephalus hexagonus* N=23) collected in veterinary clinics from several regions of France. We used diagnostic PCR to estimate the frequencies of the following symbiotic bacterial taxa: (i) the *Midichloria mitochondrii* symbiont; (ii) the *Arsenophonus*-type symbiont; (iii) the *Wolbachia* symbiont; (iv) the *Spiroplasma* symbiont; (v) the *Cardinium* symbiont; (v) the *Coxiella*-type symbiont; (vi) *Rickettsia* species; as well as the following tick-associated pathogens: (i) *Babesia*; (ii) *Borrelia* sp.; and (iv) SFG *Rickettsia* sp. Using community ecology statistical methods, we analyzed patterns of association among microbial taxa, and the effects of both tick host species and geographical factors on the distribution of tick infection. We used phylogenetic methods to analyze the diversity and relationships between strains of the inherited endosymbiotic bacterial community in ticks.

3211

Containment of artemisinin resistance in Southeast Asia - how to ensure that progress towards malaria elimination does not falter

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Asia has fought resistance to one drug after another from the 1970s. Research in recent years has confirmed an increased parasite clearance time on the Thai- Cambodian border. The biggest problem is knowing how far it has spread, but we cannot wait for more information without response, so a special containment programme is underway in the areas where there is evidence. The programme objectives are:
• To detect all malaria cases (including among mobile/migrant populations) and ensure effective treatment and *Plasmodium falciparum* gametocyte clearance.
• To decrease drug pressure for selection of artemisinin resistant parasites by improving access to appropriate treatment and preventing use of monotherapy and substandard drugs in both public and private sectors
• To prevent transmission of artemisinin resistant malaria parasites among target populations (including mobile/migrant populations) by mosquito control and personal protection
• To support containment of artemisinin resistant parasites through comprehensive behavior change communication (BCC), community mobilization, and advocacy
• To provide effective management (including information systems and surveillance) and coordination to enable rapid and high quality implementation of the strategy
Key areas of focus are
1. Mobile and migrant populations, who have limited access to control services but potential to spread resistant parasites to new areas. The programme explores how to reach these people, including new economic migrants;
2. Surveillance and information systems, which need to be rapidly upgraded to detect hotspots in transmission, to capture areas with higher frequency of slow parasite clearance and to be complete and timely for rapid response;
3. Suppression of monotherapies
4. Private sector strategies to ensure more rational drug use;
5. Understanding patient behaviour to support changes which will limit risk of spread;
6. Joint action by Thailand and Cambodia
Conclusion. Extraordinary efforts are needed to control malaria even where it is less common, but there will be beneficial side-effects in improving surveillance and learning for elimination. A major question is whether we are missing the main target, as information on drug efficacy is patchy.

3212

Systemic Sodium Stibogluconate Treatment of Cutaneous Leishmaniasis; Clinical Outcome and Adverse Events with Ten and Twenty Day Regimens

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Background: Pentavalent antimonials, such as sodium stibogluconate (SSG) are widely used in the treatment of New World cutaneous leishmaniasis (CL); there is less data reported using them systemically for Old World CL. We discuss the clinical outcome and adverse event profile from our experience with a large cohort treated with SSG. **Methods**: American military personnel with parasitologically confirmed CL were treated with intravenous sodium stibogluconate (Pentostam™, GlaxoSmithKline, United Kingdom) 20mg/kg/day under IND#14150. Treatment duration was generally 20 days but in a subset of those with nonfacial, uncomplicated *L. major* we used a 10 day course. Participants were seen daily during the treatment period and then provided follow up information at about 2, 6, and 12-24 months after treatment. Clinical cure was defined as the patient considered their lesions healed at 6 months with no reactivation up to 12-24 months. Data are given as intent to treat analysis. **Results**: 414 CL patients were treated with SSG, 141 prescribed 10 day (SSG 10), 273 had 20 day (SSG 20) drug courses. The median number of skin lesions was 3 (range 1-32) and 98% of infections were due to *L. major*. 96% of SSG 10 and 85% of SSG 20 completed the prescribed duration of SSG. Adverse events led to treatment discontinuation in 5 (4%) SSG 10 and 39 (15%) SSG20. Overall adverse events included blood disorders 22%, EKG changes 29%, gastrointestinal symptoms 73%, arthralgias 69%, herpes zoster 2%, elevated ALT 59%, elevated lipase 80%. Adverse event intensity (assessed by functional impact) was mild in 76% and severe in 3%. At six months 97(86%) SSG 10 and 200(91%) SSG20 ($p>0.05$), and at 12 months 100% SSG 10 and 99% SSG 20 reported being healed. 37(9%) received additional subsequent treatments (thermotherapy, cryotherapy, azoles, imiquimod, repeat antimonial course, amphotericin); 57% re-treatments were in the SSG10 group. **Conclusions**: In this mainly *L. major* CL cohort, both 10 and 20 days of intravenous sodium stibogluconate were associated with clinical cure in 86%, 91% respectively at six months. Adverse events occurred commonly but were predominantly graded as mild in intensity. Our experience suggests that systemic SSG can be used safely to treat Old World cutaneous leishmaniasis.