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PRESUMPTIVE TREATMENT INCREASES RISK OF INFLAMMATION AND POOR FETAL OUTCOMES IN WOMEN WITH PLACENTAL MALARIA AT DELIVERY

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Intermittent preventive treatment during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) has been shown to reduce the incidence of malaria-associated poor pregnancy outcomes. However, parasite resistance to SP is a growing problem world-wide and is particularly widespread in Muheza, Tanzania. Our previous work in Muheza indicates that IPTp selects for placental infections with increased levels of resistance, decreased diversity, increased parasitemia, and increased inflammation. Inflammatory infiltrates and cytokines have been associated with poor outcomes, particularly among first time mothers. We have now assessed the effect of IPTp on outcomes in women without (PM-) versus with placental malaria (PM+) at delivery. Among PM- women, IPTp was associated with an increase in cord and placental inflammatory cytokines, but was nevertheless associated with improved birth weight outcomes. Among PM+ women, IPTp was associated with increased histologic evidence of inflammation, as well as decreased fetal hemoglobin, increased risk of fetal anemia, and worsened birth weight outcomes. Independent of the effects of IPTp use, the parasite resistance allele at codon 581 in DHPS was associated with decreased fetal hemoglobin, increased risk of fetal anemia, and decreased birth weight. Thus, where IPTp use may have prevented infection (*ie*, PM- women) outcomes were improved, but where it failed to prevent infection (PM+ women) disease was exacerbated, compared to women who did not use IPTp. We hypothesize that IPTp may worsen outcomes in a subset of women via its separate effects on inflammation and resistance, and that as drug resistant parasites spread in a community the proportion of women harmed by IPTp will exceed the proportion benefited.

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RETINAL ANGIOGRAPHIC CHANGES IN PAEDIATRIC CEREBRAL MALARIA

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The retina contains the only part of the central nervous system vasculature that can be directly examined during life. Characteristic changes can be seen in the retina of children with cerebral malaria (CM). Fluorescein angiography permits direct and repeated observation of the patency, contours and continence of retinal vessels. This allows close investigation of a malaria infected neurovasculature. The objective of this study was to assess the retinal circulation in children with CM. With parental informed consent we performed retinal fluorescein angiograms (FA) on 82 Malawian children admitted with CM who had retinopathy determined on ophthalmoscopy. Extent of retinal non-perfusion was classified by extent in optic disc areas (DA). Repeat examinations were performed if possible. Vessels seen as orange in colour on ophthalmoscopy appeared on angiography to be patent but to contain luminal irregularities and filling-defects, these changes resolved by 24 hrs. Vessels seen as white, (which sometimes had previously been orange), were commonly non-patent and were associated with areas of retinal non-perfusion. 11/82 patients had severe non-perfusion (≥ 5DA): 1 died and 5 had gross neurological sequelae on discharge. Of 71 patients with lesser or no non-perfusion 6 had neurological sequelae Central areas of non-perfusion resolved within 4 days. In 5 patients angiography demonstrated marked and continuous leaking of fluorescein. 4 of these 5 patients died. In conclusion, retinal intravascular filling defects may result from the presence of parasitized red blood cells, with or without accompanying platelets and microthrombi. If non-perfusion occurs in the brain it is a possible mechanism for neurological sequelae, suggesting possible new avenues of therapeutic study. Leaking of fluorescein from retinal vessels indicates breakdown of the blood:retina barrier; similar change in the brain may contribute to raised intracranial pressure observed in paediatric CM.

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TRANSCRIPTIONAL PROFILE COMPARISON OF HOST IMMUNE RESPONSE TO SEVERE VERSUS UNCOMPLICATED *FALCIPARUM* MALARIA: A CASE-CONTROL STUDY IN MALIAN CHILDREN

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The clinical outcome of infection by *Plasmodium falciparum*, the parasite most often responsible for severe malaria, is varied and complex, ranging from asymptomatic parasitemia to death. To elucidate the potential protective host factors that govern the spectrum of clinical response to *P. falciparum* infection, we are measuring human gene expression in blood samples collected as part of a case-control study in children under the age of 5 years in Bandiagara, Mali, an area of intense seasonal transmission of malaria. From September to December 2008, severe malaria cases (n=24) were selected based on the World Health Organization definition of severe malaria, and uncomplicated malaria controls (n=23) with fever greater than 37.5°C and *P. falciparum* parasitemia were selected and matched according to age, location and ethnicity. Blood was collected at at the time of presentation prior to administration of medication, a week following presentation, and during the dry season (March 2009) when seasonal transmission of malaria is extremely low. Extracted RNA from the samples is currently being used to create transcriptional profiles using whole human genome expression arrays on the Affymetrix GeneChip® platform in Bamako, Mali. Results are pending owing to ongoing microarray processing and data analysis and will be presented in November.

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DOWN-REGULATION OF ANTI-INFLAMMATORY AND ANTI-APOPTOTIC GENE EXPRESSION DURING UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Our previous studies have demonstrated up-regulation of genes associated with the innate immune response, inflammation, complement-related and apoptosis pathways during the acute illness associated with uncomplicated *Plasmodium falciparum* malaria. Although we had postulated that up-regulation of genes associated with the immune response and apoptosis should be linked to down-regulation of genes with opposing effects, those genes were not identified initially because the algorithms focused primarily on increased gene expression during the acute illness. Immune-modulatory genes which were down-regulated initially, which then returned to normal with clinical recovery (days 7-10) included the MHC Class II antigens DOα

and DOβ, cell differentiation antigens CD6 and CD1c, and transcription factor 7. Anti-apoptotic genes that were down-regulated initially which likewise returned to normal by days 7-10 included c-Myc, TNF receptor superfamily member 25 (TNF R SF25), interferon regulatory factor (IRF4), KBBKG, caspase 8, and Fas Ligand G. These findings indicate that the human response to uncomplicated *P. falciparum* malaria includes both the up-regulation of immune response, inflammation, complement-related and apoptotic genes and the concomitant down-regulation of genes with opposing effects. They suggest that the successful human response to uncomplicated *P. falciparum* malaria involves more than up-regulation of inflammation and complement-related pathways; that it may also involve (perhaps require) linked down-regulation of genes with opposing effects in order to produce a successful integrated host response.

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DETECTION AND VALIDATION OF COMPLEMENT COMPONENT C3A AS A NOVEL BIOMARKER FOR CEREBRAL MALARIA

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Biomarkers that enable early and accurate diagnosis of individuals at risk of developing severe manifestations of *Plasmodium falciparum* infection would facilitate clinical management and allow for more efficient allocation of health resources. To this aim, we used Surface-enhanced laser desorption time of flight mass spectrometry (SELDI-TOF-MS) on fractionated mouse serum from resistant and susceptible mouse strains throughout the course of infection with *Plasmodium berghei* ANKA to identify early biomarkers of cerebral malaria. Complement component C3a, a key protein involved in innate immunity and inflammatory response to infection, was among the set of candidate biomarkers identified. Levels of C3a were significantly higher in susceptible mice as early as one day post infection, and persisted throughout the course of infection ($p<0.01$). The expression differences of C3a in response to *P. berghei* ANKA infection were confirmed in sera from a second set of susceptible versus resistant mouse strains ($p<0.05$). To validate the clinical utility of C3a as a diagnostic biomarker for CM, we examined levels of C3a in plasma samples from *Plasmodium falciparum*- infected patients with uncomplicated (UM) or cerebral malaria (CM). C3a levels were significantly increased in CM versus UM ($P<0.05$) and naïve samples, indicating that C3a may represent a clinically informative biomarker for severe malaria.

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WHOLE BLOOD ANGIOPOIETIN-1 AND -2 LEVELS DISCRIMINATE CEREBRAL AND SEVERE MALARIA FROM UNCOMPLICATED MALARIA

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Severe and cerebral malaria are associated with endothelial activation. Angiopoietins are critical regulators of endothelial quiescence and recent data suggests they may be informative biomarkers in severe infections. We examined the utility of whole blood angiopoietin (ANG) levels to discriminate between uncomplicated (UM, n =70), cerebral (CM, n =87), and severe (non-cerebral, n =36) *Plasmodium falciparum* malaria (SM) in Thai adults. Receiver operating characteristic (ROC) curve analysis demonstrated that the level of ANG-1, the level of ANG-2 or the ratio of ANG-2: ANG-1 was able to discriminate between individuals with UM and SM (area under the curve (AUC), p-value: ANG-2, 0.763, $p<0.001$; ANG-1, 0.884, $p<0.001$; ANG-2: ANG-1, 0.857, $p<0.001$). Similarly, all markers discriminate between UM and CM (AUC, p-value: ANG-2, 0.772, $p<0.001$; ANG-1, 0.778, $p<0.001$; ANG-2: ANG-1, 0.820 $p<0.001$). Further, ANG-1 was able to discriminate between SM and CM (AUC, p-value: ANG-1: 0.735, $p<0.001$). The ability of the angiopoietins to differentiate between the different clinical syndromes was independent of covariates (age, gender, ethnicity, parasitemia), as determined by multivariate logistic regression. The sensitivity and specificity were calculated for each biomarker, and revealed that ANG-1 was best able to discriminate between UM and SM (sensitivity: 86.1%, specificity: 85.7%); whereas ANG-2 was better at discriminating between UM and CM (sensitivity: 75.9%, specificity: 77.1%). The combined ratio of ANG-2: ANG-1 was best at discriminating between UM and complicated malaria (SM and/or CM) (sensitivity: 78.9%, specificity: 82.9%). Overall, these results suggest that whole blood ANG-1/2 levels are promising clinically informative biomarkers of severe malarial syndromes. Finally, their robust detection in whole blood makes ANG-2 and ANG-1 attractive candidates for incorporation into point-of-care testing.

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THE ROLE OF BOVINES IN HUMAN *SCHISTOSOMA JAPONICUM* INFECTION IN THE PEOPLES' REPUBLIC OF CHINA

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Zoonotic schistosomiasis japonica is a major public health problem in China. Bovines, particularly water buffaloes, are thought to play a major role in the transmission of schistosomiasis to humans in China. Preliminary results (1998-2003) of a praziquantel (PZQ)-based pilot intervention study we undertook provided proof of principle that water buffaloes are major reservoir hosts for *S. japonicum* in the Poyang Lake region, Jiangxi Province. Here we present the results of a cluster-randomised intervention trial (2004-2007) undertaken in Hunan and Jiangxi Provinces, with increased power and more general applicability to the lake and marshlands regions of southern China. The trial involved four matched pairs of villages with one village within each pair randomly selected as a control (human PZQ treatment only), leaving the other as the intervention (human and bovine PZQ treatment). A sentinel cohort of people to be monitored for new infections for the duration of the study was selected from each village. Results showed that combined human and bovine chemotherapy with PZQ had a greater effect on human incidence than human PZQ treatment alone. The results from this study supported by previous experimental evidence, confirms that bovines are the major reservoir host of human schistosomiasis in the lake and marshland regions of southern China, and reinforce the rationale for the development and deployment of a transmission blocking anti-*S. japonicum* vaccine targeting bovines.

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EVIDENCE OF SYNERGISTIC EFFECTS BETWEEN *PLASMODIUM* SPP. AND *SCHISTOSOMA HAEMATOBIUM* INFECTIONS ON ANEMIA AND STUNTING IN KENYAN CHILDREN

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Concomitant infections with multiple parasites are widespread throughout the developing world, especially in tropical climates. Despite this reality, detailed estimates of associated morbidities are lacking. This cross-sectional study based in Kingwede, Kenya investigated associations between parasitic infections (*Plasmodium* spp. and *Schistosoma haematobium*) and morbidities, including anemia and stunting, in 318 children aged 8-17. Results of multivariable general estimating equation (GEE) analyses accounting for household clustering of participants showed significant sex and age differences in the prevalence of these morbidities. Specifically, girls aged 12-17 (n=82) experienced the highest prevalence of anemia (>30%) whereas boys of the same age (n=80) were most likely to be stunted (>35%). In addition, important associations were found between heavy infections and anemia and stunting in girls; girls with heavy *Plasmodium* infections (categorized based on results of semi-quantitative PCR analyses) were 3.17 (95% CI: 1.42-7.10) times more likely to be anemic than were those with no or light infections. Similarly, girls with heavy *S. haematobium* infections (≥100 eggs per 10mL urine) were 2.46 (95% CI: 1.02-5.94) times more likely to be stunted than were those with no/light infections. Finally, male children burdened with heavy concomitant infections had lower blood hemoglobin and lower height-for-age z-scores than did those who were uninfected or lightly infected. This trend was not seen when heavy single-species infections and uninfected/lightly infected were compared. Results suggest a synergistic relationship between *Plasmodium* and *S. haematobium* parasites in their effects on morbidities, at least among male children. Integrated control efforts targeting multiple parasite infections in school-aged children could enhance efforts to reduce anemia and stunting in this population.

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THE IMPACT OF COMMUNITY CHARACTERISTICS ON INDIVIDUAL INFECTION RISK: NIGHT SOIL USE AND SCHISTOSOMIASIS TRANSMISSION IN SOUTHWEST CHINA

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While individual behaviors may increase a person's risk of infection, such as water contact in the case of schistosomiasis, the activities of others may also impact on an individual's infection risk. For example, the use of human waste as an agricultural fertilizer may facilitate the spread of pathogens to farmers on neighboring fields. Traditional models examining individual-level risk factors overlook important exposure pathways defined by the activities of others. We examined the impact of community and household night soil use on schistosomiasis in 53 villages in Southwest China where schistosomiasis reemerged after declining below detectable levels. We tested 2839 people for *S. japonicum* infection using the Kato-Katz and miracidial hatch tests, collected demographic data from each participant, and interviewed the head of each household about agricultural practices, socio-economic indicators, and other infection risk factors. Each household reported the number of buckets of night soil applied to crops in the past year. Village night soil use was defined as the average night soil use by households outside of each person's home. Models adjusted for within village correlation using generalized estimating equations. Most households (57%) reported using human waste as fertilizer in the past year. Human infection prevalence (6.7% overall) varied widely by village (0 to 42.9%). Greater village night soil use was associated with a significantly higher risk of infection (OR 1.9, 95% CI: 1.1 - 3.3), controlling for household night soil use, age, SES and county of residence. Household night soil use was not associated with infection. Night soil use by community members outside of the household is an important determinant of schistosomiasis infection risk in reemerging areas. Models of schistosomiasis and other environmentally mediated infectious diseases must consider not only individual-level risk factors but the important role community-level risk factors play in determining infection risk.

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A SURVEY OF RODENTS FOR SCHISTOSOMES FROM THE LAKE VICTORIA BASIN, KENYA, THE DISCOVERY OF A NEW SCHISTOSOME SPECIES, AND THE IMPLICATIONS FOR SCHISTOSOMIASIS CONTROL STRATEGIES

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Kisumu is a Kenyan port city, located on the eastern shore of Lake Victoria. The lake's abundant snail populations coupled with high levels of contamination and the intimate contact of residents with the lake have led to consistently high prevalence rates of intestinal schistosomiasis. Recent schistosomiasis control efforts, both in Kisumu and elsewhere in Africa, have focused nearly exclusively on treatment of humans with praziquantel. However, the extent to which wild animals act as reservoirs for *Schistosoma mansoni* and could serve as a source of renewed transmission following control efforts is poorly known. With the objective to study the role of small mammals as reservoir hosts, more than 500 animals belonging to 12 rodent and one insectivore species were examined for infection with schistosomes around Kisumu. Animals were collected from 2 types of sites: near the lake shore and from a swamp draining into the lake. About 4% of the animals contained schistosomes, including several murid rodent species and one species of shrew. Four schistosome species were recovered: Schistosoma mansoni, S. bovis, S. rodhaini, and a previously undescribed species. This new species is morphologically similar to members of the Schistosoma haematobium group, but differs from them by producing relatively small Schistosoma intercalatum-like eggs with a relatively small length to width ratio. Comparison of DNA sequences strongly supports the status of these worms as a new species and as a sister species of S. intercalatum. Although this new species has only been found in rodents, phylogenetically it falls in the middle of a clade of schistosomes which includes members infective to humans. The finding of the human-infecting *S. mansoni* in these reservoir populations is significant due to the possibility they could perpetuate snail infections and favor renewed transmission to humans once control programs have ceased. Thus, long-term control strategy must consider the implication of abundant and infected reservoir hosts.

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KHAT, SNAILS AND FLUKES: CLINICAL AND EPIDEMIOLOGICAL FEATURES OF FASCIOLIASIS, AN EMERGING DISEASE IN THE UK

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A reported increase in UK veterinary fascioliasis led to enhanced surveillance for human cases. We report 13 cases in England and Wales between January 2008 and March 2009, compared to 6 in the preceding decade. 7 were diagnosed at one centre, after an initial case raised

awareness. We also report the first indigenously acquired case for at least 10 years. We suggest that human fascioliasis is increasing and under-diagnosed in the UK. We reviewed recent cases to identify common features. Cases were identified by the national reference laboratory for *Fasciola* serology. The most common features were eosinophilia (11/13), abdominal pain (9/13), fever (6/13), and deranged liver function with raised alkaline phosphatase predominant (9/13). Imaging was abnormal in 10/13 cases, normal in 2, and is pending in 1. The nature of abnormalities varied, but most were heterogeneous lesions on ultrasound. Other findings included serpiginous lesions on MRI, tubular hypodensities on CT, a lesion in the hepatic vein, and a mass lesion. 9 of 13 were migrants from the Horn of Africa and Yemen; 6 admitted to using khat, a leaf from the shrub *Catha edulis* chewed for psychoactive properties. Khat is imported fresh from Africa, provides an ideal environment for *Fasciola* metacercariae, and is used exclusively by this migrant population. Of the others, one was Zimbabwean, and two were returning travellers from Africa. 12 of 13 cases were therefore imported, either due to acquisition of the parasite abroad or consumption of an imported crop. Infection in one case was attributed to wild Welsh watercress, the first indigenously acquired fascioliasis in England and Wales for over 10 years. Thus, the rise in UK human fascioliasis is largely imported by migrants and travellers from the Horn of Africa or acquired in the UK by this population from khat. This is likely to be mirrored in other high-income countries with similar migrant populations. A high index of suspicion is needed in patients with eosinophilia and appropriate risk factors, since clinical and imaging features are not uniform.

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ACCESSIBILITY AND UTILIZATION OF SCHISTOSOMIASIS-RELATED HEALTH SERVICES IN A RURAL AREA IN NORTHERN MINAS GERAIS STATE, BRAZIL

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The objective of the present study was to compare access to and utilization of schistosomiasis diagnostic and treatment services in a small village and the surrounding rural area in northern Minas Gerais State, Brazil. This study enrolled 1,228 subjects, including 935 residents of the central village, and 293 in rural area. *S. mansoni* infection rates were significantly higher in the central village (44.3%) than in the rural area (23.5%) during both our survey in 2007 and during the 2002 schistosomiasis case finding campaign (30.1% and 24.3%), (P<0.001). However, only 23.8% of the villagers and 26.8% of the rural residents obtained tests on their own during the 2003-2006 period from health centers, hospitals, and private clinics in various nearby towns. Results point to inequitable access and utilization of schistosomiasis diagnostic and treatment services. The main barriers associated with low utilization at the individual level were poverty, lack of knowledge by subjects regarding schistosomiasis and a high rate of home medication. At the health services level, the main barriers were failure of the local health services to routinely collect and test stool-samples for *S. mansoni* infection, the absence of treatment services outside the health center in Jequitinhonha urban area and lack of health education programs. Large distances to local health facilities, lack of public transportation and poverty contributed to the low rate of health-care utilization for the diagnosis and treatment of schistosomiasis. Even in the study area, access to the health service was higher in the central village than in the surrounding rural area (P<0.001). Thus poverty, deficiencies of the health services and accessibility problems are major factors in the persistence of hyperendemic schistosomiasis in the study area. A combination of low utilization rates after 2002 campaign until 2006 and persistence of high *S. mansoni* infection rates argue for the acceleration of the integration of the schistosomiasis control program into the primary health services. Strengthening of the primary health services, particularly laboratory and schistosomiasis treatment services, in São Pedro District can make them more accessible to the at-risk population. This must also include a health education program that focuses on health promotion and active participation of the community.

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RAPID ASSESSMENT OF *SCHISTOSOMA HAEMATOBIUM* INFECTION IN NIGER USING SCHOOL-BASED QUESTIONNAIRES

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Simple and accurate means of rapidly identifying communities at highest risk schistosomiasis is key to implementing control activities. School-based questionnaires covering symptoms of urinary schistosomiasis have been widely used to classify the endemic level in the community. On the basis of this classification the optimal frequency of treatment of school age children can be determined. The threshold for applying yearly mass treatment is visible haematuria greater than 30%; this strategy may leave many schools without treatment even though some of the children are infected. This study investigates the diagnostic performance of school-based questionnaires as a rapid and cost-effective method for estimating the prevalence of *Schistosoma haematobium* infection in seven districts of Niger. A face-to-face questionnaire about health problems including whether they had schistosomiasis as well as associated symptoms such as blood in the urine and pain during urination, was administered by teachers to a total of 9600 schoolchildren (aged 6-15 years) from 161 schools. Self-reported symptoms were then validated by screening 960 respondents for microhaematuria using reagent sticks. The prevalence of reported schistosomiasis in the interview was strongly correlated with the prevalence of infection determined by microhaematuria. This study highlights the relationship between self-reported symptoms and infection prevalence and intensity, according to age and sex. We will discuss the sensitivity and specificity of diagnosis by the interviewing tool according to varying prevalence of infection. Our findings suggest that in Niger, self-reported symptoms provide a useful rapid method for identifying communities with a high prevalence of morbidity. This inexpensive method is a potential tool for sustainable control in the context of finite resources.

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GEOGRAPHIC VARIATION IN STOCKING AND SALES OF SUBSIDIZED ARTEMISININ-BASED COMBINATION THERAPIES BY PRIVATE DRUG SHOPS IN TWO RURAL DISTRICTS OF TANZANIA

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Millions of individuals with malaria-like fevers purchase drugs from private retailers, but artemisinin-based combination therapies (ACTs), the only effective treatment in regions with high levels of resistance to older antimalarial drugs, are rarely obtained through these outlets. To encourage scale up of ACT coverage, the global malaria community is launching the Affordable Medicines Facility - malaria (AMFm) to subsidize the price of ACTs at the point of production for distribution in the public and private sectors. However, concerns remain about whether the intervention will succeed in reaching poor, rural communities. As a result, the Government of Tanzania and the Clinton Foundation piloted this subsidized distribution model in two Tanzanian districts. Data on stocking of ACTs and other antimalarials in all retail shops and health facilities in the districts were collected at baseline and in four subsequent surveys over 15 months. Exit

interviews collecting sociodemographic indicators were conducted with all customers purchasing antimalarial drugs at the shops during each survey period. All shops and facilities were georeferenced, and variables related to population density and proximity to distribution hubs, roads, and other facilities were calculated in ArcGIS. Logistic regression models were used to examine the influence of geographic and socioeconomic factors on stocking and sales of ACTs. Multivariate models indicated that although total ACT purchases rose from negligible levels to nearly half of total antimalarial sales over the course of the pilot, considerable geographic variation in stocking and sales persisted and was related to a variety of socio-spatial factors; sales were less likely in shops farther from district capitals (OR=0.97 for a 1km increase [95% CI 0.95-0.99]), major roads (OR=0.77 [0.64-0.93]), and at lower population density (OR=3.44 for each 100/km^2 [1.58-7.50]). This analysis confirms the potential for a subsidy to increase ACT usage but indicates that supporting interventions may be required to ensure access equity.

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RAPID UPTAKE OF ARTEMISININ-BASED COMBINATION THERAPY IN RUFIJI DISTRICT, TANZANIA

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In recent years, artemisinin-based combination therapies (ACTs) have been widely adopted as first-line treatment for uncomplicated malaria. While the efficacy of ACTs is well-established, little data are available to guide the scale up of this intervention. The Interdisciplinary Monitoring Project for Antimalarial Combination Therapy in Tanzania was conducted to pilot and evaluate district-wide implementation of an ACT in the Rufiji District of Tanzania. Two neighboring districts were non-intervention districts, in which sulfadoxine-pyrimethamine (SP) monotherapy was the first-line treatment per national policy. Co-packaged SP and artesunate (AS) were introduced in February 2003 in all district health facilities in Rufiji. Supporting interventions included age-specific preprinted dosing envelopes, health worker training, and job aids. Demographic and dispensing data were collected from all patients at the pharmacies of selected facilities for one month per quarter, from October 2002 - December 2005. In Rufiji, 57.0% of patients received a clinical diagnosis of malaria, compared to 46.6% in non-intervention districts ($p < 0.0001$), suggesting increased care seeking for febrile illness at health facilities in Rufiji. Of patients with a clinical diagnosis of malaria, 98.8% received an antimalarial in Rufiji compared to 95.7% in non-intervention districts. In Rufiji, 75.9% (95% CI 75.5-76.2) of patients received SP-AS during the study period, increasing from 72.7% in the first quarter to 88.1% in the final quarter. Of patients who received SP-AS, 94.6% (95% CI 94.4-94.8) received the correct dose of both. Among patients in Rufiji who received SP, 15.5% (95% CI 15.2-15.8) received SP monotherapy, and among patients who received AS, 0.25% (95% CI 0.21-.30) received AS monotherapy. The uptake of SP-AS in Rufiji was rapid and sustained. Although some SP monotherapy occurred, AS monotherapy was rare, and most received the correct dose of both drugs. These results suggest that implementation of an ACT, accompanied by training, job aids, copackaging and assistance in stock management, can rapidly increase access to effective antimalarial treatment.

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SAVING LIVES THROUGH INCREASED ACCESS TO ARTEMESIN-BASED COMBINATION THERAPY (ACT): THE EXPERIENCE IN MALAWI

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Malaria is endemic in Malawi and is the leading cause of death for children under five. Malaria cases account for 40% of outpatient care and are the largest single cause of hospital admissions (39%). The Malawi National Malaria Control Program's (NMCP) five year plan aims to reduce malaria mortality by 50% by the year 2010. One of the major interventions to achieve this goal is to expand access to ACT_the WHO approved treatment for uncomplicated malaria. The President's Malaria Initiative is supporting this effort through the provision of 15 million ACT treatments and technical assistance in case management and supply chain strengthening. In less than two years, Malawi was able to rapidly expand access to ACTs through the public sector and faith-based facilities and establish the systems to maintain a continual supply of ACTs. This was accomplished by an initial ACT distribution in October 2007, establishing an information system that provides stock status and consumption at all levels, conducting regular supervision, monitoring and adjusting procurement plans, developing tools for managing four different ACT presentations (adjust for substitution), and monitoring order fill rates. Preliminary results include: distributing 2.6 million treatments to 560 facilities in less than two weeks, identifying and responding to consumption 27% greater than forecast, and limiting stock outs of all four presentations to less than 15%. By understanding the critical success factors that contributed to Malawi's achievements, policy makers and program managers can work to strengthen their treatment programs and expand access to ACTs.

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THE COST OF SCALING UP INTERMITTENT PREVENTIVE TREATMENT IN INFANTS IN AFRICA

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Intermittent preventive treatment in infants (IPTi) is a new malaria control strategy that has been demonstrated to reduce malaria incidence during the first year of life by 30% and can be delivered with routine immunization programmes. In 2007, UNICEF launched a pilot study involving more than 270,000 children in six malaria-endemic countries in Africa to evaluate the feasibility, acceptability and the cost of scale-up IPTi under routine implementation conditions. Implementation scale-up cost was calculated for each of the six countries by estimating IPTi incremental costs in the start-up year and in subsequent years. Costs included were the financial costs of administering IPTi to children (drugs and equipment) and of programme activities (advocacy, micro-planning, programme communication, health workers training and supervision). Costs were calculated separately for the first year and for subsequent years (recurring costs). Because a survey of community and health care workers' knowledge, attitudes, and practice regarding IPTi showed high acceptability and high health care worker knowledge, recurring programme costs included only supervision. The unit cost of implementation was calculated by the weighted average of the six study countries; and by calculating an optimal weighted average after excluding three countries whose IPTi coverage was below expected levels or whose expenditures were abnormally high. IPTi incremental costs in start-up was 2.27 US$/infant and recurrent costs were 68 UScents/infant; similar results were obtained using an optimal approach (2.00 US$/infant and 66 UScents/infant respectively). During start up, programme expenditures averaged 82% of costs and training and supervision were the most expensive components, accounting for 26% of all costs. Costs of administering IPTi to children were generally very low (40 and 25 UScents/

infant in start up and recurrent years, respectively). IPTi can be scaled up successfully at very low cost. Recurrent costs are very low, especially if initial communication and training are well-conducted.

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PRE-REFERRAL RECTAL ARTESUNATE IS COST-EFFECTIVE FOR TREATING SEVERE CHILDHOOD MALARIA

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Severely ill malaria patients with vomiting, prostration and coma cannot be treated orally and require injections. In remote areas, the access to health facilities providing such parenteral treatment is limited. Hence, safe and effective treatment of most severe childhood cases is delayed or not achieved. A large-scale randomized controlled trial of children with suspected severe malaria in rural Ghana, Tanzania, and Bangladesh showed that a single dose of rectal artesunate prior to referral for standard parenteral therapy reduced mortality and permanent disability by 30%. Rectal artesunate affects the progression of the disease by rapidly reducing parasite density. We estimated the incremental cost-effectiveness of pre-referral rectal artesunate treatment followed by parenteral treatment relative to the existing practice of parenteral treatment in health facilities. Health outcomes are estimated based on the trial efficacy results and severe malaria incidence in children living under stable endemic conditions. We considered the incremental costs of providing artesunate suppositories in rural villages through a network of community health workers to a target population of 50,000 people. We also account for the initial start-up costs to establish a community-based program. We included variable costs of inpatient care as pre-referral treatment was assumed to reduce the average length of inpatient stay from 7 to 3 days among recovered patients. While taking cost savings due to a shorter inpatient stay into consideration, fixed costs of inpatient care were excluded, as these would not change due to the intervention. Assuming full coverage with pre-referral treatment at the community level, the cost ranges from $6,952 per death averted to a cost savings of $109 per death averted and from $208 per DALY averted to a cost savings of $3 per DALY averted, depending on the level of patient adherence to referral for standard parenteral treatment. Under at least moderate coverage and assuming a moderate level of adherence to referral, pre-referral treatment with rectal artesunate is a cost-effective adjunct to standard parenteral treatment of severe malaria cases, judged by a standard of under US$ 150 per DALY averted for an 'attractive' intervention. Under at least high coverage and assuming a high level of adherence to referral, the intervention is 'highly attractive' with a cut-off value of $25 per DALY averted.

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IDENTIFYING THE OPTIMAL ANTI-MALARIA PROGRAM FOR A COMMUNITY: THE NEED TO FOCUS ON SOCIAL WELFARE AS THE TARGET OUTCOME

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Although it is widely recognized that the impact of anti-malaria vector control interventions on malaria transmission differ across space and time, differences in the impact of these interventions on other aspects of social welfare are seldom identified. Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) may reduce the risk of vector-borne diseases other than malaria and environmental management may decrease cases of cholera and/or reduce damage due to flooding. Given the limited resources in the public sector in sub-Saharan Africa, the objective for all public programs should be to maximize social welfare. We defined the optimal anti-malaria program for a policy maker to implement using a given level of resources as the program which yields the highest overall social welfare impact. Using a dataset constructed from two malarious communities over a 20-year period, we developed a methodology for evaluating the impact of all feasible anti-malaria programs in each community using social welfare as the outcome of interest versus only using malaria morbidity and malaria mortality. We defined the impact of each of the feasible anti-malaria programs on social welfare as the malaria impact (morbidity and mortality), the non-malaria health impact and non-health impact. Relative to the optimal anti-malaria program we identified based on our evaluations using social welfare, we find that the current practice of using only the malaria impact as the target outcome leads to the selection of a program which was sub-optimal in terms of social welfare. These findings suggest that new frameworks are needed for identifying how to allocate scarce resources to suppress malaria transmission in sub-Saharan Africa which target improving social welfare rather than a narrow focus only on malaria.

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HOW TO ENSURE *PLASMODIUM FALCIPARUM* CHEMOSENSITIVITY RESULTS IN THE FIELD?

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To ensure comparability of different studies *Plasmodium falciparum* chemosensitivity that we performed on the field, we tested the concept of a mobile modular laboratory. This field investigation unit allows a quality of manipulations in endemic areas where no well-equipped laboratory is available similar to that found in the reference laboratory. This prototype integrates all the elements for the treatment of the samples and the parasite culture. Two specific equipments were designed to satisfy the constraints of the parasite *in vitro* culture: a trigas incubator chamber supplied with CO2 and N2 gas and a mini-class II biological safety cabinet. A colorimetric assay was chosen for testing parasite growth instead of the traditional isotopic assay unallowable in field. This lab unit was completed by a cold chain to keep the ELISA and culture reagents at +4°C during the transport. This lab was used during 2008 in the Binh Phuoc province of Vietnam, on the Kampuchea-Vietnam border. In the field, fresh isolates of *P. falciparum* were collected and were cultured using 96-well microculture plate pre-coated with drugs. *P. falciparum* isolates were successfully tested for their susceptibility to chloroquine, quinine, dihydroartemisinin, lumefantrine, piperaquine and doxycycline for 80% of samples. In conclusion, this microbiological lab allowed us to identify the drug resistance status of Plasmodium infections in the field just like in the reference lab. The modular structure of this mobile lab will permit to respond to the multiple specific needs of governmental and non-governmental organizations in the event of health crisis, emerging infectious or disaster situation.

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HOW TO GET SAFE WATER: PERSUASION, PEERS, PRICE, PROMOTERS, OR PRODUCT?

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Two million children die each year from diarrheal disease, due in part to fecal contamination of water. Chlorine treatment at the point of use can significantly cut this toll, yet less than 10% of households in our Kenyan study area regularly use individually-packaged dilute chlorine solution,

sold at a retail price of $0.30 per month, despite several years of a vigorous social marketing campaign. We explore the role of persuasion, peers, price, promoters, and the product's delivery system in boosting chlorine use through a series of randomized evaluations. More intense persuasion efforts had little impact and we do not find strong evidence of peer effects. Price seems to be the single most important factor, with more than half of people using chlorine when it is delivered free to the house. Locally-elected chlorine promoters can boost chlorination rates, and are effective even when compensated at a flat rate alone rather than via bonuses for good performance. Based on the evidence, we developed a chlorine dispenser technology which drastically reduces the cost of chlorination and leads to the highest rates of use. Because the cost of the packaging is high relative to the cost of the chemical, supplying a community with bulk chlorine through a dispenser is vastly cheaper than subsidizing individually-packaged bottles. In addition, the dispenser provides a physical reminder to treat water at the moment when it is most salient (as water is collected), is more convenient to use than bottled chlorine, and maximizes the potential for peer effects by making each household's decision of whether or not to treat their water public, allowing community members to help one another learn to use the technology and set an example for others to follow. Chlorine dispensers are a promising new technology for providing access to safe water where piped infrastructure is not available. We estimate that the cost per DALY saved by a chlorine dispenser could be less than $20, extremely cost-effective relative to other public health interventions in less-developed countries.

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INTERIM ASSESSMENT OF A SANITATION, HYGIENE EDUCATION AND WATER SUPPLY INTERVENTION IN RURAL BANGLADESH, 2008

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The Sanitation, Hygiene Education and Water Supply in Bangladesh Programme (SHEWA-B) is a large intensive program focusing mainly on social mobilization for awareness building, targeting 30 million rural population. The programme aims to improve standards of hygiene practices and behavior, whilst ensuring adequate sanitation and safe water supply. To measure early effects of the intervention in the programme areas, we conducted an interim assessment, at least 6 months after implementation, using 5-hour structured observations of hand washing behavior and spot-checks of water sources, latrines, and waste disposal. These data were compared to those collected at baseline from the same 500 households, selected randomly. At interim assessment, the proportion of people washing both hands with soap or ash after defecation increased to 30% compared with 17% at baseline, and after cleaning a child's anus the proportion rose to 34% compared to 22% at baseline. There were no changes in hand washing practices for food related events. Improved latrine coverage defined by WHO/UNICEF Joint Monitoring Programme increased to 91% in comparison to 88% during baseline. Open defecation declined from 10% to 8%. Child's feces were disposed of properly in 11% of households, a slight increase over 9% at baseline. Availability of appropriate household solid and liquid waste disposal was low, less than 3%, both at interim assessment and at baseline. About 99% households use protected source of drinking water both at interim assessment and at baseline. In conclusion, from the findings of the interim assessment we can conclude that additional time is required to observe further behavior change for some indicators linked to the study. While there have been improvements for some indicators, this assessment only collected information from the intervention households. Nevertheless, these data are useful interim indicators of the study progress and are helpful in identifying areas for improvement in the project implementation cycle.

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A COMPARISON OF WATER TREATMENT PRACTICES AMONG PEOPLE LIVING WITH HIV/AIDS AND COMMUNITY MEMBERS IN ETHIOPIA, DECEMBER, 2008

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Diarrhea is a leading cause of illness and death in people living with HIV/AIDS in sub-Saharan Africa. Chlorinating household drinking water reduces the risk of diarrhea in this population by at least 25%. In Ethiopia, some HIV programs promote and distribute WuhaAgar, a socially marketed water chlorination product. We assessed these programs by comparing WuhaAgar use among HIV-infected antiretroviral treatment (ART) clinic clients and community members. We surveyed 795 clients from 20 ART clinics and 795 community members (matched by age, sex, and neighborhood) about water-handling practices and WuhaAgar use. We tested stored household drinking water for residual chlorine. Clinic clients were more likely than community members to report chlorinating household water (30% vs. 8%, matched odds ratio [mOR] = 5.5; 95% confidence interval [CI]: 3.9-7.9), have a WuhaAgar bottle at home (21% vs. 3%, mOR = 8.7; 95% CI: 5.3-15.1), and have chlorine residuals in stored water (7% vs. 1%, mOR = 13.3; 95% CI: 4.9-50.4). In a subset analysis, clients from five clinics that promoted WuhaAgar were more likely than clients of clinics that did not promote WuhaAgar to report water chlorination (52% vs. 13%, adjusted odds ratio [aOR] = 7.1; 95% CI: 2.7-18.9) have WuhaAgar at home (36% vs. 9%, aOR = 6.1; 95% CI: 2.5-14.9), and had a greater tendency to have chlorine residuals in stored water (11% vs. 4%, aOR = 3.3; 95% CI: 0.9-11.9). In conclusion, ART clinic clients were more likely than community members to use a socially marketed water chlorination product. Promoting and distributing water chlorination products in ART clinics may increase water treatment and prevent diarrhea among people with HIV/AIDS.

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RANDOMIZED CONTROLLED TRIALS OF A PLASTIC HOUSING BIOSAND FILTER IN CAMBODIA, GHANA AND HONDURAS

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Point of use (POU) drinking water treatment allows people without access to safe water sources to improve the quality of their water by treating it in the home. One of the most promising emerging POU technologies is the biosand filter (BSF). Field studies of the concrete-housing BSF in Cambodia and the Dominican Republic have documented significant reductions in diarrheal disease and continued use over time. As a result of these and numerous other studies, there is growing interest in scaling up POU technologies. An alternative to the typically cumbersome concrete BSF is a plastic-housing one, one of which is a plastic BSF of the NGO International Aid. The purpose of this research was to document the ability of this BSF to reduce diarrheal disease in user households compared to non-user households. In 2008, three randomized controlled trials (RCTs) of plastic BSFs were performed in Cambodia, Ghana and Honduras. Approximately 150-250 households were recruited from rural villages in each location. Households were randomized to the plastic BSF intervention or no intervention at the village level in Cambodia and Ghana and at the household level in Honduras. Households were observed during the intervention period for four to six months. Initial results indicate significant reductions of diarrheal disease in Cambodia and Ghana with households reporting approximately 60% fewer cases of diarrheal disease. In Honduras, plastic BSF households also experienced considerably decreased

rates of diarrheal disease compared with non-filter households but initial analysis suggests this reduction not to be statistically significant. The observed reduction in diarrheal disease associated with plastic BSFs in all locations was within the range reported for other POU technologies such as the concrete BSF, chlorine disinfection or ceramic filtration. Hence, a plastic housing BSF appears to be an effective household POU water treatment technology that is physically easier to implement than the comparable concrete versions.

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A RETROFIT TO UPGRADE LOW-COST CERAMIC WATER FILTER DEVICES TO PURIFIER STATUS

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Ceramic candles have earned a place in household drinking water treatment in many countries, lending themselves readily to gravity-feed applications, and improving the safety and appearance of the filtered water. Steady improvements in ceramic technology have led to advances in performance that, at the high end, include effective removal of cysts and bacteria. At the low end, lesser efficacies are the rule, with variable and inconsistent removal of cysts and bacteria, depending on porosity and local manufacturing standards. We have developed a simple retrofit device aimed at upgrading the decontamination efficacy of existing ceramic-based filters to include disinfection of water borne viruses, at the high end, and enabling lower quality products to achieve superior bacterial removal. Customized cartridges containing variable amounts of brominated hydantoinylated polystyrene beads (EPA Reg#.72083-3) were prototyped with a view to incorporating a quick-fit housing containing the medium directly into the water treatment train. Water exiting the ceramic element passes through the bead bed, and is decontaminated largely by contact, with the added benefit that suitably prepared beads could also impart a valuable but imperceptible halogen residual to the stored product water. Microbial challenges of the filters with polio, MS-2, *E.coli* and *K.terrigena*, and determination of the efficacy of the residual, showed that retrofitted devices of a variety of brands in India were able to provide long-lasting purification levels with attractive cost-benefit features. Results of Indian consumer reactions to the retrofit approach will be presented, as well as data on a lower- capacity, 2 gal. plastic device designed for service as an emergency relief purifier.

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HOUSEHOLD PREDICTORS OF ABUNDANCE OF THE LASSA VIRUS RESERVOIR, *MASTOMYS NATALENSIS*, IN THE EASTERN PROVINCE OF SIERRA LEONE

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Lassa fever is a severe hemorrhagic illness caused by Lassa virus, which is transmitted to humans primarily by contact with the excreta or blood of *Mastomys* rodents. Current recommendations to reduce *Mastomys* abundance in homes include improved food storage and removal of rodents by trapping or poison. It is unknown if house construction influences *Mastomys* infestation or if improved house construction is an effective control. For this study, two villages (<100 houses) and two towns (>200 houses) were sampled during the dry season in the Eastern Province of Sierra Leone, an area of high Lassa fever incidence. Household features including construction type and refuse location were documented, and knowledge of Lassa fever was assessed in one resident per household. To date, 142 houses have been sampled 102 in villages, where all homes were included, and 40 in towns, where homes were sampled in two transects along the town perimeter. Three of the study sites had a laboratory-confirmed case of Lassa fever within two weeks prior to site visit, and rodent tissue was collected for further studies. A total of 224 small mammals were trapped in 2,909 trap nights (overall trap success [TS]=7.7%), including 162 *M. natalensis* (*M. natalensis* TS=5.6%), 55 *Rattus*, 6 *Crocidura*, and 1 *Mus*. Absolute number and TS of *M. natalensis* was similar between villages and towns. In a linear regression model, *Mastomys*-specific TS was correlated with the number of rodent burrows observed in homes (t=2.62, p=0.01). Wall and roof type were not associated with *M. natalensis* TS, however, type of floor construction did approach significance (t=-1.84, p=0.068). This relationship may be elucidated as we accrue a larger sample size in this ongoing study. *Mastomys* are burrowing rodents and the number of burrows observed may serve as a rapid indicator of *Mastomys* abundance and Lassa fever risk, as suggested by previous investigators. The ability of *Mastomys* to burrow in dirt floors may determine their capacity to infest houses, and promotion of cement floors may be a valuable control measure.

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ANTIMALARIAL ANTIBODIES ARE GOOD MARKERS OF PRIOR EXPOSURE BUT NOT PROTECTION AGAINST SUBSEQUENT MALARIA IN CHILDREN IN KAMPALA, UGANDA

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Individuals in malaria-endemic regions develop antibodies against multiple parasite antigens, but associations between antibody levels and protection against subsequent malaria remain uncertain. We enrolled a cohort of 438 children aged 1-10 from a region of Kampala where malaria incidence was previously found to be heterogeneous, with those living near a swamp having 4 times the incidence of those living 200 meters away. Children were treated for all episodes of malaria and monitored every 30 days for asymptomatic parasitemia. Plasma samples were collected at least 180 days after enrollment. For children with at least 1 year of follow-up after sample collection, IgG responses were assayed via ELISA to *P. falciparum* circumsporozoite protein (CSP), liver-stage antigen 1 (LSA1), merozoite surface protein 3 (MSP3), 3 variants of merozoite surface protein 1 (MSP1), and 2 variants of apical membrane antigen 1 (AMA1). Reponses to different antigens were analyzed for associations with antecedent environmental and host factors, and for associations with subsequent malaria incidence. Overall antibody prevalence ranged from 12% (CSP) to 29% (AMA1). Increasing age, residence within 25 meters of the swamp and a shorter interval between last documented parasitemia and the time of plasma collection were all significantly associated with higher levels of antibodies to all 5 antigens in multivariate analysis. Higher antibody levels significantly predicted higher incidence of subsequent malaria after adjustment only for age (incidence 6%-16% higher per doubling of antibody level), but not after adjustment for age, prior malaria incidence, and distance from the swamp. In summary, IgG levels to 3 blood-stage and 2 pre-erythrocytic antigens were all markers of prior parasite exposure, but they did not predict protection against subsequent malaria. The relationship between antimalarial antibodies and subsequent malaria incidence may be confounded by heterogenous exposure to parasites.

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ANALYSIS OF THE BIOLOGICAL FUNCTION OF ANTIBODIES ELICITED FOLLOWING IMMUNIZATION OF MALARIA-NAÏVE SUBJECTS WITH A *PLASMODIUM FALCIPARUM* ERYTHROCYTE-BINDING ANTIGEN 175 (EBA175) VACCINE

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Erythrocyte-binding antigen 175 (EBA-175) is a *Plasmodium falciparum* erythrocytic-stage protein which binds to glycophorin A on the surface of the erythrocyte during the process of merozoite invasion. A subregion of EBA175 - Region II - has been identified as a candidate for a blood-stage malaria vaccine since it contains this sialic acid binding domain. Non-glycosylated recombinant EBA-175 RII formulated on Adju-Phos® was evaluated in a U.S. Phase 1 double-blinded dose-escalation trial. Four groups (20 subjects each) received either placebo (2 per group) or increasing doses of the EBA175 vaccine (range= 5ug to 160 ug) at 0, 1 and 6 months. Sera were analyzed by ELISA to characterize humoral immune responses. Significant increases in ELISA titers were seen in the EBA175 RII-vaccinated groups as compared with the day 0 samples, although there were no significant differences among vaccinated groups. In addition, total IgG was purified from the sera on study Days 0 and 194 (two weeks after the last vaccination) and analyzed both by ELISA and by a standardized *in vitro* parasite Growth Inhibition Assay (GIA). A low but statistically significant level of growth inhibition was observed in the day 194 samples relative to Day 0 IgGs from the same individuals, revealing *in vitro* functional activity of the elicited antibodies. We further showed that the recombinant EBA175 RII antigen binds to normal red blood cells (RBC). Selected high titer IgGs from immunized subjects collected at day 194 were shown to inhibit this RBC binding, whereas the day 0 IgGs from the same individuals did not inhibit RBC binding. Additional GIA studies have been performed to dissect the ligands in various *P. falciparum* strains that are inhibited by these antibodies. Moreover, we have shown that this antigen is recognized by antisera from individuals living in malaria-endemic areas of both Mali and Cambodia when tested by ELISA. These serological studies support further clinical development of this formulation and evaluation of other vaccine components which could be added to it.

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DIFFERENT T EPITOPE REGIONS OF THE *PLASMODIUM FALCIPARUM* MSP1-33 CRITICALLY INFLUENCED THE RESPONSIVENESS, MAGNITUDE, AND QUALITY OF ANTI-MSP1-19 ANTIBODIES

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The C-terminal fragment of the *P. falciparum* Merozoite Surface Protein 1 (MSP1-42) is a leading blood-stage malaria vaccine candidate. MSP1-33, the N-terminal processed fragment of MSP1-42, is rich in T cell epitopes. It is hypothesized that one or more of these epitopes play a role in enhancing the antibody response toward MSP1-19. In this study, we seek to provide *in vivo* proof that regions of MSP1-33 rich in T cell epitopes can indeed provide functional help in inducing anti-MSP1-19 antibodies. A total of fourteen constructs consisting of truncated MSP1-33 segments linked to MSP1-19 were expressed in *Drosophila* S2 cells and immunogenicity studies were performed in outbred Swiss Webster mice. Recombinant MSP1-19 and MSP1-42 were used as controls. Analysis of the development of anti-MSP-19 antibody responses in the outbred mice demonstrated striking differences in the helper function of the truncated MSP1-33 regions, despite the fact that they all possess T cell epitopes. A number of these fragments were highly efficacious in inducing a generalized antibody response (100% responding) among the outbred population. The immunogenicity of some of these constructs were either comparable to or surpassed the response observed with MSP1-42. These truncated MSP1 C-terminal antigens were down selected based on their responsiveness and further evaluated in outbred New Zealand White rabbits, using Montanide ISA51 as the adjuvant. High titers of anti-MSP1-19 antibodies were observed in animals immunized with the truncated antigens. Animals immunized with a subset of truncated antigens developed potent parasite growth inhibitory antibodies, while others were ineffective despite very high antibody titers. Efficacious fragments were further examined in terms of their ability to prime for antibody responses to the full length MSP1-42. Results of these ongoing studies, including analyses of antibody specificity, will be presented. The significance of these data in relation to the development of a more potent vaccine based on the MSP1 C-terminal regions will be discussed.

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PFEMP1 IS THE MAJOR TARGET OF ANTIBODIES TO THE SURFACE OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES THAT ARE ASSOCIATED WITH PROTECTION FROM MALARIA

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Parasite-derived variant surface antigens (VSA) are expressed on *Plasmodium falciparum*-infected erythrocytes (IEs) and are major targets of immune responses. Antibodies to VSAs develop in a variant-specific manner and are associated with protection from symptomatic and severe malaria. Several confirmed or proposed VSA have been identified, including PfEMP1 (*P. falciparum* erythrocyte membrane protein 1), rifin, STEVOR, and SURFIN protein families, and others. However, the significance of many of these proteins as targets of acquired antibodies remains unclear. Dissecting which VSAs are antigenically dominant has been limited until now by a lack of specific tools. In this study, we have used parasite lines in which PfEMP1 surface expression was inhibited by transfection of parasites with a construct that suppresses endogenous var gene promoter expression. We developed assays to measure PfEMP1-specific antibodies by comparing antibody reactivity to the IE surface of transfected versus parental parasites. Using this approach with samples from Kenyan children and adults suggests that PfEMP1 is the major target of antibodies to the IE surface in the great majority of individuals. In most samples, antibodies to PfEMP1 accounted for 80% or more of the total antibody binding to IEs. PfEMP1-specific antibodies were associated with age and active parasitemia, and prospectively associated with reduced risk of symptomatic malaria. These findings provide strong evidence that PfEMP1 is the dominant VSA and that it is an important target of protective immunity. This has significant implications for understanding acquired immunity to malaria in humans.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE ON IMMUNE RESPONSES TO ERYTHROCYTIC STAGE ANTIGENS IN MOZAMBICAN CHILDREN

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Intermittent preventive treatment in infants with sulfadoxine-pyrimethamine (IPTi-SP) alongside the EPI, has shown to be an efficacious and safe intervention against malaria in sub-Saharan Africa, and is currently being considered for adoption as policy. However, data on the effect of IPTi on the development of naturally acquired immunity is scarce. In the context of a randomized, placebo-controlled trial of IPTi in Mozambique we have recently assessed antibody responses to whole asexual parasites by IFAT and to recombinant merozoite antigens by ELISA. Further evaluation of the impact of this intervention on the development of antibody responses as well as cellular responses to *Plasmodium falciparum* is needed. We evaluated the impact of IPTi-SP, given at 3, 4, and 9 months, on the development of antibody and cellular responses to *P. falciparum* in Mozambique. In a group of 302 infants at ages 5, 9, 12, and 24 months, we measured IgG against VSA and growth inhibitory antibodies were measured at ages 12 and 24 months both using FACs. Intracellular and extracellular cytokines were measured by FACs and luminex respectively. Preliminary data suggest that cytokine responses do not significantly vary in children receiving SP or placebo. Results on the effect of IPTi on the development of IgG to VSA and on growth inhibitory antibodies will also be presented. In conclusion, based on previous assessment of antibody responses and on preliminary results on cellular responses, IPTi-SP does not appear to negatively affect the development of immune responses to *P. falciparum* and, in some cases, it appears to be associated with higher antibody levels.

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ACQUISITION OF ANTIBODIES TO THE PFRH2 INVASION LIGANDS OF *PLASMODIUM FALCIPARUM* AND THEIR ASSOCIATION WITH PROTECTION FROM MALARIA

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Repeated exposure to *Plasmodium falciparum* eventually results in protective immunity in humans that protects from symptomatic malaria and high density parasitemia. Antibodies to *P. falciparum* play an important role, but their targets have been poorly defined, and antibody effector mechanisms are not well established. Antibodies may act by targeting erythrocyte invasion ligands expressed by the merozoite form of parasites. *P. falciparum* has been shown to use different subsets of erythrocyte invasion ligands to facilitate immune evasion and adapt to host receptor polymorphisms. *P. falciparum* reticulocyte binding homologues (PfRh1, PfRh2, PfRh4, and PfRh5) are important invasion ligands and are potential vaccine candidates. PfRh2 is present as two forms, PfRh2a and PfRh2b, and plays an important role in sialic acid independent invasion of red blood cells. However, the importance of PfRh proteins as targets of acquired immunity has not been established, and currently there is no data examining the association between antibodies to any of the PfRh proteins and protective immunity. In this study, we generated recombinant proteins covering the whole sequence of PfRh2a and PfRh2b. Antibodies to these proteins and a range of other merozoite antigens were assessed among a longitudinal cohort of 200 children in Papua New Guinea. Antibodies to all regions of PfRh2 were strongly associated with protection from symptomatic malaria and a reduced risk of high density parasitemia. The PfRh2 specific response appears to be predominantly of IgG1 and IgG3 sub-types. Furthermore, we have sequenced regions of PfRh genes from the same population and found that there is significant polymorphism in one region of the protein, suggesting it may be under selective pressure. Our results provide important insight into the development of naturally acquired immunity and will contribute to improved vaccine design.

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IDENTIFYING B-CELL EPITOPES WITHIN THE LIGAND DOMAIN OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN

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The Duffy binding protein of *Plasmodium vivax* is vital for host erythrocyte invasion and the region II (DBPII) contains critical residues for receptor recognition thereby making the molecule a good vaccine candidate for vivax malaria. Although an ideal target, the allelic variation within the DBPII and associated strain specific immunity is a major challenge for development of a broadly effective vaccine for vivax malaria. To understand the specificity of protective immune response to DBPII, we have generated a panel of monoclonal antibodies (MABs) to identify and map the various domains of the DBPII that correlate with protection to *P. vivax.* Using rDBPII from different alleles we have assayed the specificity of the MABs by ELISA and inhibition of binding by standard erythrocyte binding assay. Analysis by ELISA show that some of the MABs react strongly with some rDBPII alleles but not with others, while some other MABs react with all the rDBPII alleles. Preliminary analysis with erythrocyte-binding inhibition assays in which some of the MABs, showed little or no inhibition, are similar to the ELISA results. These data are consistent with the differential inhibitory pattern observed with human sera from individuals naturally exposed to *P. vivax*. Identification of conserved inhibitory epitopes to which inhibitory antibodies bind is critical to optimizing DBP immunogenicity for protection against diverse *P. vivax* strains.

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ASSESSMENT OF FIELD SITES FOR CLINICAL TRIALS OF A NEW MALARIA VACCINE IN AFRICA

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Evaluation of potential field sites is critical to planning clinical evaluation of malaria vaccines in endemic areas. To assess potential field sites for clinical trials of a live, attenuated sporozoite vaccine, we drew from experience of other groups to develop a site assessment tool. We used this tool to evaluate sites in four African countries. The site assessment team included representatives from the INDEPTH-Malaria Clinical Trials Alliance, PATH Malaria Vaccine Initiative (MVI), U.S. Navy Medical Research Unit 3 Detachment Accra, Ghana, and the Howard Hughes Medical Institute / Center for Vaccine Development at the University of Maryland. Using other site assessment/evaluation tools, including the MVI and

Pediatric Dengue Vaccine Initiative formats, a site assessment tool was developed with a consultant who specializes in international clinical trials in developing countries. This tool included evaluation of previous trial experience, personnel, subject population, potential recruitment/ cultural/community issues, institutional and national ethics and pharmacy board review processes, regulatory issues, physical space, pharmacy and laboratory capacity, data management capabilities, referral hospital(s), and transportation issues. A schedule of 1½ to 2 days at each site was planned at a time convenient for the sites and the site assessment team. Four sites were assessed using pre-determined criteria. The site assessment tool was partially completed by the Principal Investigator at each site before the site assessment team's arrival. The site assessment team was able to efficiently evaluate the suitability of each site for phase 1, 2 and 3 clinical trials and report results to the clinical development team. The site assessment process is a critical step in planning the clinical development of any vaccine. Using a standard site assessment tool adapted for the specific vaccine permits systematic and objective evaluation of potential sites for a vaccine trial. Other groups planning clinical trials in developing countries can benefit from using this method.

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RANDOMIZED, CONTROLLED, PHASE 2B CLINICAL TRIAL TO EVALUATE THE SAFETY, IMMUNOGENICITY AND EFFICACY OF WRAIR'S AMA-1 MALARIA VACCINE (FMP2.1) ADJUVANTED IN GSK BIOLOGICALS' AS02A VS. RABIES VACCINE IN 1-6 YEAR OLD CHILDREN IN BANDIAGARA, MALI

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The malaria vaccine candidate antigen FMP2.1 is a recombinant protein based on the 3D7 strain *Plasmodium falciparum* apical membrane antigen-1 (AMA-1). The purpose of this randomized, controlled Phase 2 clinical trial (NCT: 00460525) is to evaluate the safety, immunogenicity and efficacy of FMP2.1 formulated in GlaxoSmithKline's Adjuvant System AS02A in children in Bandiagara, Mali, West Africa. Four hundred healthy children aged 1-6 were randomized 1:1 to receive three doses of 50 µg of FMP2.1 in 0.5mL of AS02A or rabies vaccine, 30 days apart. The primary efficacy endpoint is time to first or only clinical malaria episode occurring between randomization and six months after the third immunization. Secondary endpoints include incidence density of clinical malaria episodes, time to first or only clinical malaria episode caused by parasites with AMA-1 genotypes identical to the 3D7 vaccine strain with respect to designated polymorphic codons, and asexual parasite density. Titers of anti-FMP2.1 antibody will be measured by ELISA. Data from the time of enrollment through six months after the third immunization will be unblinded and analyzed while the study continues in a single-bind fashion. In May-July of 2007 745 children were screened and 400 enrolled, of whom 377 (94%) received all 3 immunizations. Safety, efficacy and immunogenicity results will be presented. In conclusion, if this trial demonstrates efficacy against genetically diverse parasites and acceptable safety and tolerability, further clinical development can be envisioned, either alone or as part of a multi-stage, multi-antigen malaria vaccine.

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IMPACT OF *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN 1-COMBINATION 1/ALHYDROGEL VACCINE ON GROWTH-INHIBITORY ACTIVITY OF ANTIBODIES IN CHILDREN IN MALI

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A Phase 2 clinical trial was conducted with *Plasmodium falciparum* Apical Membrane Antigen 1-Combination 1 (AMA1-C1) on Alhydrogel® in healthy children 2-3 years old living in Bancoumana, Mali. A total of 300 children received either the study vaccine or the comparator. Although the study vaccine induced anti-AMA1 antibodies in vaccinees, no impact was seen on clinical protection against malaria. In this study, we performed Growth Inhibition Assay (GIA) with protein-G-purified IgGs from plasma collected before and after vaccination (Day 0 and Day 42) to evaluate whether vaccination with AMA1-C1 induced biologically active antibodies. In a previous study in Malian adults, vaccination with AMA1-C1 induced significantly higher antibody titers but did not increase GIA activity. In this study, there was a low but significant increase in GIA activity: the median change in% inhibition between Day 0 and 42 for the AMA1 group was 9% and for the comparator groups was -4% (Mann-Whitney test, $P<0.0001$). There was a significant correlation between anti-AMA1 ELISA titer and the GIA activity (Spearman Rank test, $r_s=0.675$, $p<0.0001$). However, of 485 samples tested only ~7% of IgGs had more than 50% inhibition. To test the specificity of GIA activity, we performed antigen-reversal GIA with selected IgGs which showed more than 50% inhibition. When IgGs with high GIA activity on Day 0 (thus induced by natural infection rather than vaccination) were tested, the activity was not reversed by preincubation of test IgGs with AMA1 protein. On the other hand, when IgGs from the AMA1 group with low GIA and ELISA titer on Day 0 but high GIA and ELISA titer on Day 42 were tested, the activity of Day 42 IgG was reversed by preincubation with AMA1 protein. These data suggest that although the GIA activity of total IgGs is correlated with the anti-AMA1 ELISA titer of the samples, only GIA activity induced by vaccination can be reversed by AMA1 preincubation; growth inhibition induced by natural infection cannot.

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A PHASE 1 TRIAL OF A BIVALENT MSP2 BLOOD-STAGE MALARIA VACCINE FORMULATED WITH MONTANIDE ISA 720

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In a previous Phase 1/2b trial carried out in Papua New Guinea, an experimental malaria vaccine containing the *Plasmodium falciparum* blood-stage antigen MSP2 reduced parasite densities in children ages six to nine by 62%. Genotyping of breakthrough parasitaemias indicated that the vaccine protected against parasites of the 3D7 MSP2 genotype corresponding to the vaccine antigen, but not the FC27 MSP2 genotype (the other dimorphic form). Therefore a dose-escalating, double-blinded,

placebo-controlled Phase 1 trial was undertaken in healthy, malaria-naive adults to test safety and immunogenicity of a new MSP2 vaccine (MSP2-C1)containing two recombinant forms of MSP2 (3D7 and FC27), representative of the two families of MSP2 alleles. The antigens were formulated in equal amounts with Montanide ISA 720, a water-in-oil emulsion. The trial was designed to include 3 dose cohorts, each with 12 subjects receiving the vaccine in doses of 10, 40, and 80µg of vaccine and 3 subjects receiving ISA 720 adjuvant emulsion alone. Three doses were administered at 12-week intervals. Due to unexpected reactogenicity at the local injection site, which will be described, and concern regarding the vaccine stability, the trial was terminated after the second dose in cohort 2. Vaccine immunogenicity was evaluated by measuring antibody (IgM, IgG, and IgG subclasses) and T cell responses to both recombinant antigens and to native MSP2. Cytophilic IgG1 and IgG3 responses were elicited against both MSP2 isoforms and a vigorous CMI response was also seen in a proportion of vaccinees. The functional activity of the antibodies is being tested in growth inhibition and antibody-dependent cellular inhibition assays. In view of the reactogenicity of this Montanide ISA720 formulation, further clinical development of MSP2-C1 will most likely depend on formulation in an alternative adjuvant.

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NEUTRALIZING ANTIBODY TITERS TO SIMIAN ADENOVIRAL VECTORS FOLLOWING ADCH63 ME-TRAP IMMUNIZATION IN HUMANS

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Immunization regimes against *Plasmodium falciparum* malaria incorporating the antigenic epitope string ME-TRAP have shown promise in clinical trials to date with protection at the liver stage leading to significant delays to parasitaemia. The efficacy of such regimes has been limited by their ability to induce strong immunogenicity. Adenoviral vectors have generated great scientific interest in recent years for their ability to induce superior immunogenicity with great potential for vaccine regimes. Their potential use in humans, however, is limited by natural anti-vector immunity to human adenoviruses. This problem could be largely circumvented by the use of simian adenoviral vaccine vectors, such as AdCh63. Protection against *P. berghei* malaria has been seen in a murine model using a simian adenoviral vector prime (AdCh63 ME-TRAP) and modified vaccinia (MVA ME-TRAP) boost and levels of immunogenicity were higher than previously seen using other viral vector regimes. To determine the immunogenicity of such a regime in man, human volunteers were immmunized with AdCh63 ME-TRAP prime and boosted 8 weeks later with MVA ME-TRAP. In order to investigate the role of neutralizing antibodies against the simian adenoviral vector, AdCh63, neutralizing antibody titers to AdCh63 were examined prior to immunization and at regular time points following immunization. Those with high levels of neutralizing antibodies were excluded from the study. Neutralizing antibody titers were correlated with ELISPOT immune responses to the vaccine. We found little relation between low levels of pre-existing or induced neutralizing titers to AdCh63 and the immune response to the antigenic insert ME-TRAP.

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SAFETY, TOLERABILITY, IMMUNOGENICITY AND PROTECTIVE EFFICACY OF AN ADENOVIRUS-VECTORED *PLASMODIUM FALCIPARUM* MALARIA VACCINE IN HEALTHY, MALARIA-NAÏVE ADULTS

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Malaria remains one of the world's major public health problems, with a vaccine urgently needed. In work aimed at the development of a multistage vaccine, the U.S. Military Malaria Vaccine Program has evaluated two prototype adenovirus (serotype 5)-vectored vaccines. Adenovectors encode the malaria antigens *Pf*CSP (expressed in sporozoite and early liver stages) and *Pf*AMA1 (expressed in sporozoite, liver and erythrocytic stages), respectively. In a preliminary dose selection trial, two groups of volunteers (n=6/group) received the vaccines in combination in either a single $2x10^{10}$ particle dose ($1x10^{10}$ pu/vaccine) or a single five fold higher $1x10^{11}$ particle dose ($5x10^{10}$ pu/vaccine). This combination vaccine was generally well tolerated at both doses. ELISpot cell-mediated immune responses summed over 9 (CSP) or 11 (AMA) overlapping peptide pools demonstrated statistically significantly higher interferon γ ELISpot responses to the CSP antigen in the low (range: 114-1066 sfc/10^6 PBMCs) compared to the high (range: 52-493) dose group ($p<0.05$). Antibody responses induced to both antigens were low, but trended higher in response to the high dose group. Prioritizing the superior ELISpot responses, the low dose was selected for the subsequent Phase 1/2a trial, which employed the *Pf*CSP vaccine only. 14 volunteers received two administrations at the lower dose of *Pf*CSP, given 4 months apart. ELISpot responses were similar in magnitude to those seen in the dose selection trial and did not statistically differ between first (range: 71-1128) and second (range: 53-596) immunizations. Three weeks after immunization, 12 vaccinees and 6 unimmunized infectivity controls underwent *Pf* sporozoite challenge. All 12 vaccinated volunteers became parasitemic with no delay to patency relative to the six controls. Conclusions: In this regimen, (1) CSP given as the sole antigen does not confer protection in human volunteers in spite of significant ELISpot responses; (2) a second dose of adenovector does not increase ELISpot or antibody responses compared to a single dose.

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DEVELOPMENT OF A SAFE AND REPRODUCIBLE HUMAN SPOROZOITE CHALLENGE MODEL FOR *PLASMODIUM VIVAX* IN HEALTHY ADULTS IN THE UNITED STATES

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With around 70 to 80 million cases per year, *Plasmodium vivax* is the most widespread malarial infection of man, causing a highly debilitating disease characterized by multiple relapses. Increasing resistance to anti-malarials by the parasites and to insecticides by the *Anopheles* vector highlights the requirement for a *P vivax* vaccine and the urgency to develop a safe

mosquito challenge model for vaccine and drug evaluation. This study is the first experimental challenge for *P. vivax* to be conducted in the US under regulatory oversight. Objective: To conduct a proof-of-concept clinical investigation to develop a safe and reproducible sporozoite challenge model for *P. vivax* in humans with a goal of 100% infectivity rate. Methods: Because *P. vivax* has proved nearly impossible to culture *ex vivo*, blood from naturally-infected volunteer-donors from a malaria clinic at Mae Sod, Thailand, an area of known chloroquine-sensitive *P. vivax*, was used for membrane feeding to clean, laboratory-colonized *Anopheles dirus* mosquitoes from AFRIMS insectary. The blood will be screened negative for potential confounding pathogens including other malaria species, filariasis, Japanese encephalitis, chikungunya, HIV, hepatitis B and C. Mosquito batches with highest oocyst infection and only those that have fed on blood negative for these pathogens will be transported to the WRAIR insectary for challenge. Two cohorts involving six healthy US volunteers each will be challenged via mosquito bites to demonstrate reproducibility of the challenge procedure. The first cohort will be given 5 infected bites; if 100% volunteers develop infection, the same will be repeated in the second cohort. If less than 100% volunteers are infected, the second cohort will be given up to 10 infected bites. Volunteers will be monitored for development of parasitemia by daily blood smears from day 5 to day 19 and closely observed in a hotel from day 10 up to about day 19, followed by periodic visits until the final visit at 6 months post challenge. Infected volunteers will be treated with chloroquine and primaquine by direct observation therapy with follow-up to document resolution of all symptoms and elimination of parasites. Safety data, prepatent period, relapse rate, and parasite genotype data from the summer 2009 challenges will be presented.

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BASOPHILS AUGMENT PARASITE ANTIGEN-SPECIFIC CD4$^+$ T CELL PROLIFERATION AND EOSINOPHILIA IN A MOUSE MODEL OF FILARIASIS

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Filariae, like other helminths, induce a type 2 immune response characterized by eosinophilia, high levels of IgE and increased T cell production of type 2 cytokines. This type 2 response is helpful in controlling helminth infections as elimination of IL-4 production or blockade of IL-4 receptor signaling increases the susceptibility of mice to infection with filariae. Previously, we have demonstrated that basophil release of IL-4 occurs shortly after the onset of detectable parasite antigen-specific IgE and appearance of circulating microfilaria in mice infected with the rodent filaria *Litomosoides sigmodontis*. Since basophils have been shown *in vitro* to be capable of inducing naïve CD4$^+$ T cells to differentiate into type 2 CD4$^+$ T cells and to induce isotype switching in B cells to IgE, we hypothesized that basophils serve to augment antigen-specific Th2 responses in murine filariasis. To test this, we evaluated type 2 immune responses in wild-type and basophil-depleted mice during chronic infection with *L. sigmodontis.* Treatment with Ba103, a monoclonal antibody that recognizes CD200R3, resulted in basophil depletion, but it did not affect peritoneal mast cells. Our data show that basophil depletion was associated with a statistically significant decrease in the ability of CD4$^+$ T cells to proliferate in response to LsAg. This decrease in CD4$^+$ T cell proliferation was not due to an intrinsic defect in proliferative ability as there was no difference in response to anti-CD3/anti-CD28 stimulation. Basophil depletion also results in decreased LsAg-driven CD4$^+$ T cell production of both Th1 and Th2 cytokines. In addition, a statistically significant reduction of circulating eosinophils was observed in basophil-depleted mice compared to wild-type mice. Our results suggest basophils amplify the immune response during infection with *L. sigmodontis* by inducing eosinophilia and CD4$^+$ T cell proliferation and cytokine production in response to parasite antigen.

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INCREASED POPULATIONS OF CIRCULATING MYELOID AND PLASMACYTOID DENDRITIC CELLS IN PATENT FILARIAL INFECTIONS

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Wuchereria bancrofti, *Brugia malayi*, and *B. timori* are the causative agents of human Lymphatic filariasis, a mosquito-borne disease which is a major cause of morbidity in tropical and subtropical regions of the world. Asymmptomatic microfilaremia, or patent infection, is a subclinical manifestation of this disease which is closely associated with an observed lack of antigen-specific T cell proliferation and production of IL-2 and IFN-γ. One proposed mechanism underlying this downregulated T cell responsiveness is antigen presenting cell dysfunction. Previously, we have shown that live microfilariae of *B. malayi* induce a caspase-dependent apoptosis in human monocyte-derived dendritic cells (DC) *in vitro*. Our present study aims to address whether apoptosis observed *in vitro* is reflected in the number of circulating myeloid DC (mDC) and plasmacytoid DC (pDC) in the peripheral blood of infected microfilaremic individuals. Utilizing four color flow cytometric analysis based on the expression of CD11c and CD123 to identify DC subpopulations, our data suggest that there was a significant increase in the number of both mDC (CD11c$^+$CD123^{lo}; geometric mean (GM) 11,000 cells/ml p=0.008) and pDC (CD11c$^-$CD123^{hi}; GM=2300 cells/ml p=0.01) per ml of blood in infected individuals when compared to the mDC (GM= 5,340 cells/ml) and pDC (GM=991 cells/ml) of uninfected controls from the same filarial-endemic region of Mali. The number of total DC, monocytes, and lymphocytes did not differ between the two groups. Using a microarray analysis to compare gene expression patterns from whole blood of these two patient groups, we observed a significant increase in the expression of COX15 and COX7A2L, cytochrome c oxidases, in microfilaremic patients compared to uninfected controls. Additionally, co-culture of patient sera with monocyte-derived DCs yielded no significant difference in cell death when comparing the two groups. Whether this increased number of mDC and pDC is a function of high turnover to replace apoptotic cells is under study.

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BASOPHILS AND MAST CELLS BECOME HYPORESPONSIVE IN CHRONIC HELMINTH INFECTIONS

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Both helminth infections and atopic diseases are characterized by increases in IgE and Th2 cytokines. However, populations infected with helminths have decreased rates of atopy. In this study, we tested the hypothesis that basophils and mast cells, effector cells of allergic responses that are stimulated through IgE, become harder to activate during chronic helminth infections. BALB/c mice were infected with *Litomosoides sigmodontis*, a filarial nematode of rodents. Peripheral blood and pleural mast cells from uninfected, acutely infected, and chronically infected mice were then stimulated with increasing concentrations of anti-IgE and evaluated for basophil and mast cell activation. Basophil activation was determined by measuring expression of surface CD200R and intracellular IL-4 using multicolor flow cytometry and by assessing IL-4 release by ELISA. Mast cell activation was determined by histamine release utilizing a competitive

ELISA. For basophils, the amount of anti-IgE required for initial as well as maximal activation became greater over the course of infection, resulting in right-shifted activation curves (mean anti-IgE concentration for maximal activation from uninfected mice = 0.017 vs. 0.446 µg/ml from chronically infected mice, $p < 0.001$ based on IL-4% positivity and 0.031 vs. 0.406 µg/ml, $p < 0.05$ based on CD200R% positivity). Importantly, over time basophils also became less responsive to IgE-independent activation by ionomycin (mean percentage of IL-4 positive basophils from uninfected mice = 89.4% vs. 79.9% from chronically infected mice, $p = 0.033$), suggesting that decreases in releasability were not due to fluctuations in surface expression of IgE. All IL-4 flow cytometry data were confirmed by ELISA. Additionally, basophils from chronically infected mice released less IL-4 when stimulated with *L. sigmodontis* antigen compared to mice that were acutely infected (mean IL-4 release from acutely infected mice = 1101.8 vs. 361.2 pg/ml from chronically infected mice, $p = 0.0076$). Mast cells from the pleural cavity, where adult worms reside, showed similar decreases in releasability as assessed by histamine release. These results indicate that basophils and, to a lesser extent, mast cells become less responsive over the course of chronic helminth infection. These findings may explain why individuals from areas with high prevalence of helminth infections have low rates of allergic disease.

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PRE-EXISTING FILARIAL INFECTION INFLUENCES CYTOKINE PRODUCTION DURING ACUTE MALARIA IN CHILDREN AND YOUNG ADULTS IN A COENDEMIC REGION OF MALI

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Filariasis and malaria infection are co-endemic in many regions of the world, including subsaharan Africa. We have previously shown that pre-existent filarial infection does not influence susceptibility to malaria infection, but may lower the threshold parasitemia at which clinical symptoms occur. To determine whether the observed decrease in clinical threshold is accompanied by alterations in the cytokine response to acute malaria infection, blood samples were collected from 16 filaria-positive (FP) and 20 filaria-negative (FN) children and young adults (1-21 years of age) who presented with clinical malaria, defined as signs and symptoms consistent with malaria infection in the presence of malaria parasitemia. Plasma levels of IFN-γ, IL-1α, IL-1β, IL-1Ra, IL-6, IL-8, IL-10, IL-12p70, IP-10 and TNF-α were measured by multiplex assay (Luminex™). Interestingly, neither the level of malaria parasitemia, nor temperature, was correlated with increased levels of pro-inflammatory cytokines. Plasma levels of IP-10 and IL1Ra, two cytokines that are strongly associated with clinical severity in malaria, were decreased in the FP subjects (GM 2130 and 82 pg/ml, respectively) during acute clinical malaria as compared to the FN subjects (GM 5308 and 489 pg/ml; $p = 0.001$ and 0.003, respectively). All other cytokine levels measured were comparable between the two groups. These findings are consistent with prior findings demonstrating a decrease in the *in vitro* production of IP-10 by PBMC from FP individuals in response to malaria antigen and provide further *in vivo* evidence that pre-existent filarial infection can modulate the immune response to incoming malaria parasites.

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PATENT FILARIAL INFECTION MODULATES THE QUALITY OF T CELL RESPONSES TO MALARIAL ANTIGENS IN MALARIA/ FILARIAL CO-ENDEMIC VILLAGE OF MALI

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Having shown that filarial infection modulates the malaria-specific pro-inflammatory cytokine response in filarial/malarial coinfected individuals, we sought to assess the frequency and the quality of the T cell populations underlying this modulation. Whole blood samples from individuals with Wb and/or Mp infections (Fil+; n=18) and those with no evidence of active filarial infections (Fil-; n=17) were stimulated with Pf-infected erythrocyte lysate [MalAg], *Brugia malayi* adult antigen (BmA), SEB, or medium for 24hours. Brefeldin A was added in the last 12h of culture, the cells were lysed, fixed and cryopreserved at -80oC until used in multiparameter flow cytometry. Filarial infection was associated with a higher frequency of CD4+ cells producing IL-4, IL-10 and IL-17 ex vivo; however, in response to MalAg stimulation, Fil+ individuals had significantly lower frequencies of IFN-γ-, IL-17- and TNF-α- producing CD4+ cells, but significantly higher frequencies of CD4+IL-10+ cells. Most importantly, in response to MalAg stimulation, Fil+ individuals had significantly lower frequencies of multifunctional T cells than did Fil - individuals ($p < 0.01$). Importantly, Fil+ individuals had no Th1 triple cytokine producers and lower frequencies of Th17 triple, double and single cytokine producers than did Fil- subjects, with no differences seen between the two groups in the proportion of multifunctional T cells in response to BMA, PPD and SEB. Analyses of regulatory T cell populations showed, in addition, that the Fil+ group had greater frequencies of CD4+CD25+Foxp3+CD127- cells as well as CTLA-4- and/or IL10-producing CD4 cells in both unstimulated and MalAg-driven cells compared to the Fil- group with major regulator, IL-10, being produced primarily by CD4+CD25- T cells and not by the nTregs. Together these data demonstrate that filarial infection induces a regulatory environment dominated by CD4+CD25- IL10 producing T cells that modulates the number and quality of Pf-specific Th1 and Th17 cells. This spillover regulation by filarial infection may have serious consequences in the clinical expression of malaria in areas co-endemic for filarial infections.

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ATTENUATION OF TLR EXPRESSION AND FUNCTION IN LATENT TUBERCULOSIS BY COEXISTENT FILARIAL INFECTION WITH RESTORATION FOLLOWING ANTIFILARIAL CHEMOTHERAPY

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Mycobacterium tuberculosis (Mtb) and filarial coinfection is highly prevalent, and the presence of filarial infections may regulate the immune response needed to control Mtb infection. Since patent lymphatic filariasis is associated with the downregulated expression and function of Toll-like receptors (TLR) and since control of Mtb is dependent on the initiation of immune responses by TLR, we sought to determine the impact of co-existing filarial infections on TLR expression and function in latent tuberculosis (TB). By analyzing the baseline and mycobacterial Ag stimulated expression of TLR1, 2, 4 and 9 (in individuals with latent TB with or without filarial infection) by real-time RT-PCR, we were able to demonstrate that filarial infection, coincident with Mtb, significantly diminishes both baseline and Mtb antigen specific TLR2 and TLR9

expression (2.65 to 7.1 fold in TLR2 and 3.72 to 3.31 fold for TLR9). In addition, pro-inflammatory cytokine responses, including IL-1b, TNF-a, IL-6, IL-12p70 and IFN-g, to TLR2 and 9 ligands are significantly diminished in filarial-TB coinfected individuals compared to individuals with latent TB alone. Pro-inflammatory cytokine production in response to TLR4 ligand stimulation (used as a control TLR stimulus) is not altered in co-infected individuals. Definitive treatment of lymphatic filariasis significantly restores the pro-inflammatory cytokine responses in individuals with latent TB (ranging from a 2 to 10 fold increase for the different cytokines) at one year post antifilarial therapy. Thus, coincident filarial infection exerted a profound inhibitory effect on protective mycobacteria specific TLR mediated immune responses in latent tuberculosis and suggests a novel mechanism by which concomitant filarial (and other systemic helminth) infections could predispose to the development of active tuberculosis in humans.

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HELMINTH-MEDIATED PROTECTION AGAINST AUTOIMMUNE DIABETES IN NOD MICE IS NOT DEPENDENT ON A TH2 IMMUNE SHIFT

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A broad range of helminths have been shown to have beneficial effects on autoimmune diseases. A leading hypothesis to explain this phenomenon is that helminth-induced type 2 immune responses limit pathogenic Th1-driven autoimmune responses. To investigate this hypothesis, the effects of *Litomosoides sigmodontis* infection on the development of Type I diabetes was evaluated in IL-4$^{-/-}$ nonobese diabetic (NOD) mice and compared with immunocompetent (WT) NOD mice. Infection of WT NOD mice with the filarial nematode *L. sigmodontis* prevented the onset of diabetes and was associated with a Th2 shift in cytokine production with significantly increased amounts of IL-4 and IL-5 from anti-CD3/anti-CD28 stimulated spleen and pancreatic lymph node cells. Significantly increased production of insulin-specific IgG1, but not insulin-specific IgG2c, showed that this Th2 shift was also present in response to one of the main autoantigens in diabetes. In contrast, IL-4$^{-/-}$ NOD mice failed to develop a Th2 shift during *L. sigmodontis* infection. Compared to WT mice, IL-4$^{-/-}$ NOD mice did not develop detectable IgE during infection, had decreased levels of insulin-specific IgG1 ($p<0.05$), and increased levels of insulin-specific IgG2c ($p<0.001$), suggesting a Th1 shift in response to insulin. In addition, infection of IL-4$^{-/-}$ NOD mice resulted in no increase in splenocyte production of IL-5 or IL-13 compared to uninfected IL-4$^{-/-}$ NOD mice and in significantly lower concentrations compared to infected WT NOD mice. As expected, splenic and pancreatic lymph node IFNγ concentrations were higher in IL-4$^{-/-}$ NOD mice compared to both infected and uninfected WT NOD mice. Interestingly, numbers of splenic CD4^{+} CD25^{+}FoxP3^{+} cells were increased in both WT and IL-4$^{-/-}$ infected mice. Despite the absence of a Th2 shift, infection of IL-4$^{-/-}$ NOD mice with *L. sigmodontis* prevented the onset of diabetes in all mice studied (n=11) whereas uninfected IL-4$^{-/-}$ controls (n=33) developed diabetes at comparable rates (67% at week 24) as WT NOD mice (n=26, 85% at week 24). These studies demonstrate that infections with filarial worms can protect against the onset of Type 1 diabetes in NOD mice by a mechanism that is independent of the host's ability to induce a Th2 shift, possibly through induction of immunoregulatory mechanisms. These results suggest it may be possible to develop worm-derived therapies for autoimmune diseases which do not induce pro-allergic Th2 responses.

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INDOOR USE OF CARBAMATE TREATED PLASTIC SHEETING IN COMBINATION WITH LONG LASTING INSECTICIDAL NETS TO CONTROL PYRETHROID RESISTANT MALARIA VECTORS IN WEST AFRICA

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Recent findings in Benin showed that pyrethroid resistance in *Anopheles gambiae* can reduce the efficacy of insecticide treated nets (ITN) and indoor residual spraying (IRS) recommended for malaria vector control. In this context, we have tested a new strategy based on a combination of long lasting insecticidal net (LLIN) and carbamate treated plastic sheeting (ITPS) to improve personal protection and "killing effect" against pyrethroid resistant mosquitoes. Experimental huts trial according to WHO phase II procedures was carried out in "Vallée du Kou" (Burkina Faso) where *An. gambiae* M and S molecular forms are sympatric and exhibit high level of pyrethroid resistance. Efficacy of LLIN (PermaNet® 2.0) alone and either in combination with ITPS (bendiocarb, 400 mg/m²), or in combination with IRS (bendiocarb, 400mg/m²) were compared in phase II trial. 1,374 *An. gambiae* were collected during the 2 months of evaluation. The blood feed inhibition was 43.4%, 58.1%, 56.3% with LLIN, LLIN+ITPS and LLIN+IRS respectively, suggesting that LLIN remains effective in term of personal protection against pyrethroid resistant mosquitoes. Low mortality rates were observed with the LLIN (44.0%), IRS (42.4%) and ITPS (52.5%) whereas both combinations killed significantly more mosquitoes (72.6% and 66.4% for LLIN+ITPS and LLIN+IRS). The results suggested that the association LLIN+ITPS (or LLIN+IRS) is a promising alternative to control pyrethroid resistant mosquitoes. A phase III trial is currently evaluating this strategy at community level in Benin to assess the people acceptability and the efficacy of these combinations on entomological, parasitological and clinical parameters of malaria.

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MULTIPLE INSECTICIDE RESISTANCE AMONG *ANOPHELES GAMBIAE* IN URBAN AGRICULTURAL AREAS OF COTONOU, BENIN (WEST AFRICA)

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Current vector control strategies rely on insecticide-treated nets (ITNs) and indoor residual spraying (IRS). However, the development of insecticide resistance constitutes a major threat to both ITN- and IRS-based control programs. Rapid urbanization without proper roads or drainage systems for rainwater, plus intensive agriculture practices, such as market-gardening, provide favorable conditions for the development of *Anopheles* mosquitoes and urban malaria. The objective of this study was to assess the status of insecticide resistance among *An. gambiae* sl populations in Cotonou, the growing economic capital of Benin. *Anopheles* breeding sites were sampled in urban areas, including market-garden zones, at the end of the dry season and the beginning of the rainy season in 2008. Larvae were brought back to the insectary and reared to adulthood. Bioassays with WHO diagnostic test kits were performed using pyrethroid, carbamate, organophosphate and organochlorine insecticides. *An. gambiae* mosquitoes were identified to species and to M or S molecular forms using PCR. Molecular and biochemical assays were performed to identify Leu-Phe *kdr* and *ace-1R* mutations in individual mosquitoes and to detect increases in the activity of enzymes typically involved in insecticide resistance (oxidases, esterases, glutathione-S-transferases). All

specimens examined were M molecular forms of *An. gambiae* ss except for one specimen of *An. arabiensis*. Resistance to permethrin, DDT and carbosulfan and decreased susceptibility to deltamethrin were detected in all populations of *An. gambiae* sampled. Cross-resistance to both DDT and permethrin was consistent with the high frequency (77-82%) of the Leu-Phe *kdr* mutation. Because the *ace-1R* mutation was found at low frequency (3-12%), carbamate resistance was due primarily to increased metabolism through enzymatic activity. A significant increase in the amount of oxidases and activity of glutathione-S-transferases was observed in comparison to the susceptible reference strain. These results have identified multiple insecticide resistance involving several mechanisms in the M molecular form of *An. gambiae* ss, the main malaria vector in Cotonou. The expansion of vegetable growing within urban areas probably contributed to selection pressure on mosquitoes.

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THE POTENTIAL FOR MALARIA CONTROL USING FUNGAL BIOPESTICIDES

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There is an urgent need for alternative tools to reduce reliance on chemical insecticides in contemporary malaria control programs. Here we present an overview of recent research demonstrating the potential for using biopesticides based on insect pathogenic fungi, in novel integrated strategies for sustainable control of malaria (and also other diseases such as dengue). Numerous fungal isolates have been shown to infect *Anopheles* mosquitoes via exposure to biopesticide-treated surfaces. Depending on fungal isolate, malaria transmission potential can be reduced through direct mortality (i.e. virulent isolates killing mosquitoes before they can transmit), conditional mortality (i.e. enhanced impact of fungal infection in mosquitoes carrying malaria) and/or transmission blocking (i.e. development of malaria parasites blocked in mosquitoes following fungal infection). In addition, spores have been shown to persist on treated surfaces up to 6 months and to pose minimal risk to human health. Exploration of these effects using models reveals that fungal biopesticides have the potential to cause considerable reductions in the density of malaria-transmitting mosquitoes. Together, these results point to the practical use of insect fungal pathogens within novel strategies of integrated vector management, with potential to both augment existing control measures and enhance long-term sustainability.

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INSENSITIVE ACETYLCHOLINESTERASE (*ACE.1^R*): EVENTS OF INTROGRESSION AND DUPLICATION BETWEEN THE MOLECULAR M AND S FORMS OF *ANOPHELES GAMBIAE* S.S.

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Characterization of insecticide resistance provides data on the evolutionary processes involved in the adaptation of insects to environmental changes. Understanding the dynamics and the evolution of genes associated with insecticide resistance between closely related taxa represents a great interest, in terms of understanding resistance evolution in the field. This is a key component in establishing effective long-term resistance management strategies to eventually adapt vector control. In an upstream study, the mutation G119S (generating *ace.1^R* allele) was found in both molecular forms of *An. gambiae* s.s. To establish whether the G119S mutation has arisen independently in each form or by genetic introgression, we analysed coding and non-coding sequences of *ace-1* alleles in M and S mosquitoes from representative field populations from West Africa. Our data revealed many polymorphic sites shared by S and M forms, but no diversity was associated with the G119S mutation. This indicates that the G119S mutation was a unique event and that genetic introgression explains the observed distribution of the G119S mutation within the two forms. Unexpectedly, sequence analysis of some resistant individuals revealed a duplication of the *ace-1* gene that was observed in both *An. gambiae* s.s. M and S forms. Again, the distribution of this duplication in the two forms most likely occurred through introgression. These results impacts on the question of actual levels of gene flow between the two molecular forms in tropical savannah areas. We can conclude that the G119S mutation could spread rapidly in the field and then compromise the use of organophosphate and carbamate compounds in public health while resurgence of interest in using Indoor Residual Spraying based on these molecules to control malaria vectors. This study underlines the necessity to monitor the G119S mutation in natural populations before planning and implementing malaria control programs based on the use of organophosphate and carbamate.

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CHANGES IN THE TRANSCRIPTION OF DETOXIFICATION GENES IN RESPONSE TO SELECTION WITH TEMEPHOS AND PERMETHRIN IN *AEDES AEGYPTI*

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Aedes aegypti control campaigns have relied on the use of insecticides for larvae and adult control since 1950. Unfortunately, these insecticides target one of two sites: acetyl-cholinesterase or the voltage dependent sodium channel. This target redundancy and other operational factors have allowed for the selection of insecticide resistance. Resistance mechanisms include primarily structural changes in target sites or increased metabolism by detoxification enzymes. Five *Ae. aegypti* strains from Mexico and one from Iquitos, Peru were selected with temephos and permethrin for five generations. Selection was replicated three times. Resistance ratios (LC50) for temephos selected strains increased 37 - 110 times when compare with the susceptible New Orleans reference strain. Although the Iquitos strain showed an initial LC50 lower than New Orleans, the LC50 increased 75 times after five generations of selection. Initially strains did not show altered acetyl cholinesterase in biochemical activity assays nor mutations in the Ace1 gene. Using the *Ae. aegypti* microarray detoxification chip we identified genes upregulated in the Iquitos-selected strain that belong to the carboxyl esterase (CCae5C, CCEae1C, CCEae2C, CCEae6C, AaeCOE-1, CCEae3o) and mono-oxygenase families (SOD4 and CYP4H31). Permethrin selection resulted in a 12 - 33 fold increase in LC50. Bioassays indicate that knockdown resistance (kdr) and post-exposure recovery were the major mechanisms for permethrin resistance in the Mexican strains. Kdr allele frequency (Ile1,016) increased almost to fixation after five generations of selection. We observed that kdr is dose-dependent and >25 µg permethrin per bottle will produce complete knockdown in a strain homozygous for the Ile1,016 allele. The Iquitos selected strain lacked the kdr mechanism and also the Ile,1,016 allele, however we observed recovery after permethrin exposure. We identified genes up regulated in the Iquitos selected strain that might be associated with recovery (GSTs-1, CCae2B, Perox3, CYP6Z9, CCEunk6, catalase, Aldox8 and AaeCOE-17). Further work will indicate if these genes are potential markers for insecticide resistance in order to support vector control campaigns.

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OPTIMIZATION OF A HOST-SEEKING MOSQUITO TRAP FOR INTEGRATION INTO AN *AEDES AEGYPTI* PUSH-PULL CONTROL STRATEGY

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The ability to repel mosquitoes from entering houses or increase exiting once inside will reduce indoor densities and man-vector contact within homes. However, there is concern within the public health community that vectors driven away from treated homes will simply divert to other untreated host sources within the surrounding area. In addition, as the focus of vector control strategies has been to directly reduce vector populations through the killing actions of chemicals, little is known regarding the modifications in behavioral responses of mosquitoes post-exposure to include the continued search for a host source once driven to the peridomestic environment. As part of a larger research program developing a push-pull strategy for *Aedes aegypti* control, a peridomestic host-seeking trap (BG-Sentinel™) is being optimized for its affinity to both quantify mosquito diversion and trap chemically repelled and/or irritated mosquitoes from the outdoor environment. We report on the use of *Ae. aegypti* populations of varying physiological states (age, parity and feeding status) to evaluate changes in trapping rates using a series of mark-release-recapture experiments under semi-field (screen-house) conditions. Variables of interest included: trap to mosquito density ratios; distance from a fixed release point; and exposure to sub-lethal doses of standard vector control chemicals. Results generated from these studies will guide trap configuration during proof-of-principle evaluations of the push-pull strategy under field conditions in Thailand.

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WILLINGNESS TO PAY FOR VECTOR CONTROL FOR THE ASIAN TIGER MOSQUITO IN NEW JERSEY

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Since its introduction in New Jersey in 1995, the Asian tiger mosquito's (*Aedes albopictus*) distribution in the state has increased substantially. However, few target-specific control measures have been implemented against this pest. To plan such measures, a telephone survey was conducted to ascertain how residents were prevented from enjoying outdoor activities by urban mosquitoes and their willingness to pay for more control measures. Using the contingent valuation stated preference technique, willingness-to-pay interviews were conducted. In 2008, a random sample of households in 6 study sites in Mercer (i.e., Trenton) and Monmouth Counties in New Jersey was chosen. Respondents were informed that current public spending was 25 cents per person per month (pppm). A split sample bidding technique was used to control for starting point bias with starting bids of 10, 25, or 75 cents pppm. From 157 households randomly chosen from residential lists, 49 (31%) were reached and consented to be interviewed. Interviews were conducted in English (82%) or Spanish (18%). Mosquitoes reportedly interfered with outdoor activities "somewhat" in 26% and "a lot" in 45% of respondents. Willingness to make payments to control urban mosquitoes was endorsed by 41% of respondents, of whom 85% would accept higher taxes and 15% would only make charitable contributions. Among respondents willing to pay higher taxes, the mean willingness to pay (±standard error) was $1.19±0.34 pppm. Responses ranged from $0.05 to $5.00 with a median of $0.75 pppm. No significant differences were found between counties, nor among different starting bids. Of those not willing to pay, 74% explained that they could not afford additional regular payments. Responses imply that residents would support an additional $400,000 yearly in county taxes (a 167% increase) for mosquito control. In conclusion, this mosquito interferes substantially with outdoor activities. Residents would support moderate tax increases for effective mosquito control.

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WEST NILE VIRUS GENETIC DIVERSITY AND RNA INTERFERENCE IN THE MOSQUITO *CULEX PIPIENS QUINQUEFASCIATUS*

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West Nile virus (WNV) exists in nature as a genetically diverse population of competing genomes. This high genetic diversity and concomitant adaptive plasticity has facilitated the rapid adaptation of WNV to North American transmission cycles and contributed to its explosive spread throughout the New World. WNV is maintained in nature in a transmission cycle between mosquitoes and birds, with intrahost genetic diversity highest in mosquitoes. The mechanistic basis for this increase in genetic diversity in mosquitoes is poorly understood, but may be attributable to differences in the innate antiviral immune response in birds (type-I interferon mediated) compared to mosquitoes which seem to rely on RNA interference (RNAi). In RNAi-mediated antiviral responses, virus-derived small interfering RNAs (v-siRNAs) are loaded into the RNA-induced silencing complex (RISC) and guide subsequent degradation of homologous RNA sequences. The sequence-specificity of RNAi may drive increases in genetic diversity within host cells, and consequently within hosts. To determine whether the high mutational diversity of WNV in mosquitoes is driven by RNAi, we characterized the RNAi response to WNV in the midguts of orally exposed *Culex pipiens quinquefasciatus* using high-throughput, massively parallel sequencing and estimated viral genetic diversity. Our data demonstrate that WNV infection in orally exposed vector mosquitoes induces the RNAi pathway as characterized by the presence of viral-derived small interfering RNAs (viRNAs) 21 nt. in length. The viRNAs had an asymmetric distribution spanning the length of the genome, where some regions were intensely targeted and others poorly targeted. Further, we found a correlation between regions of the WNV genome that are more intensely targeted by RNAi and the presence of nucleotide substitutions compared to weakly targeted regions. These results suggest that, under natural conditions, natural selection of WNV is stronger in regions highly targeted by the host RNAi response. Further, they provide a mechanistic basis for the relative importance of mosquitoes in driving WNV diversification.

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THE EFFECT OF MOSQUITO SALIVA ON THE INTERACTION OF WEST NILE VIRUS AND ITS VERTEBRATE HOST

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Arthropod saliva affects the pathogen-host interaction for several diverse systems. Our laboratory previously showed that mosquito saliva enhances West Nile virus (WNV) infection in mice. The goal of this study is to understand the role of mosquito saliva during WNV infection *in vivo*. We hypothesized that salivary components alter the innate immune response of the host, leading to higher viremia. We, therefore, determined the levels of type I interferon (IFN) and other cytokines in mice after WNV inoculation with and without mosquito saliva. In order to control for the amount of virus inoculated, we introduced saliva into the host by feeding uninfected Culex tarsalis mosquitoes on a confined area prior to inoculation with 105 PFU WNV by needle (spot feeding). Spot feeding stimulated significantly higher levels of type I IFN in serum than virus alone

at 24 and 48 hour post inoculation (hpi) and in the draining lymph nodes at 24 hpi. This induction is likely due to the higher viral loads in the serum and lymph nodes of spot-fed mice. In addition, spot feeding suppressed the production of interleukin 2 (IL-2) and IL-10 in WNV-inoculated mice, suggesting that mosquito saliva suppresses the immune response of the host during WNV infection. To identify the salivary components involved in these observations, we fractionated proteins from salivary gland extract (SGE) of Cx. tarsalis using a size exclusion column. We inoculated mice with WNV in the presence of different column fractions and determined viremia at 24 and 48 hpi. We found that proteins that were greater than 20 kDa enhanced WNV viremia at both 24 and 48 hpi to an equivalent level as SGE alone. Furthermore, fractions less than 20 kDa, void fractions, and heat-inactivated SGE had no effect on viremia. These results suggest that the salivary factor(s) responsible for enhancing WNV viremia is/are proteinaceous. Studies are underway to understand the mechanism(s) by which mosquito saliva modulates the immune response of the host during WNV infection.

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AVIAN HOSTS OF WEST NILE VIRUS IN PUERTO RICO

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West Nile virus (WNV) ecology in neotropical ecosystems is poorly understood, and vertebrate hosts responsible for infecting mosquitoes remain unidentified throughout the Caribbean Basin. A focus of intense transmission in the vicinity of Ceiba, Puerto Rico, during 2007 provided an opportunity to investigate the avian hosts of WNV in a Caribbean ecosystem. Following peak transmission as determined from seroconversions in sentinel chickens, we evaluated free-ranging, resident birds for abundance and prevalence of WNV infection, and identified vertebrate DNA in mosquito bloodmeals concurrently collected in the Ceiba vicinity. We used the mosquito inoculation index (*M*), calculated as the product of population, infection rate and vertebrate reservoir competence, to rank avian species' involvement as amplifying hosts for WNV. Greater Antillean grackle (*Quiscalus niger*) and gray kingbird (*Tyrannus dominicencis*) appear primarily responsible for WNV infection of mosquitoes in our 100-km^2 study site. In urban habitat, house sparrow (*Passer domesticus*) also appears important.

1097

THE IMPACT OF WEST NILE VIRUS ON THE ABUNDANCE OF NORTH AMERICAN BIRDS

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Emergence of West Nile virus (WNV) in North America has been associated with high mortality in the native avifauna. Some reports suggested that local populations of American crows (Corvus brachyrynchus), a particularly susceptible species, were severely impacted. A recent analysis North American Breeding Bird Survey (BBS) data by LeDeau et al (2007) indicated that populations of several species of birds were permanently reduced by the ongoing seasonal epizootic of WNV. Here, we present results of a longitudinal analysis of population number of birds, based on Bayesian hierarchical models. Separate models are fitted by state (CA, CO, FL, IL, LA, MA, MD, MN, SC, TN, WA) and species (American crow, American robin, bluejay, as well as eight other species). Our analysis addresses potential shortcomings of the study by LeDeau et al. Most importantly, we explicitly model the impact of WNV transmission intensity, as indicated by human neuroinvasive WNV disease, on year-to-year changes in observed numbers of birds of specific species. In addition, we allow for temporal auto-correlation of annual population numbers, as well as for a decrease in fatal susceptibility to fatal WNV infection of populations, as time since introduction of WNV increases. Results from this analysis will contribute to the ongoing discussion about the long-term impact of WNV in North America on native birds.

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WEST NILE VIRUS INDUCES MULTIPLE MATRIX METALLOPROTEINASES (MMP) IN HUMAN ASTROCYTES: ROLE IN TIGHT JUNCTION PROTEIN (TJP) DEGRADATION AND BLOOD-BRAIN BARRIER (BBB) DISRUPTION

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Although the mechanism(s) underlying the entry of WNV into the CNS are unclear, perturbation of the BBB is known to facilitate entry of virus and activated immune cells. MMPs, capable of digesting TJP and basal lamina have been implicated in the disruption of the BBB in neuroinflammatory diseases. We hypothesized that MMPs secreted by WNV-infected astrocytes, principal cells lining the BBB, will degrade TJP of the human brain microvascular endothelial (HBMVE) cells thereby opening of the *in vitro* BBB model and this process can be reversed by treatment with MMP specific inhibitors. HBMVE cells and human brain cortical astrocytes (HBCA) were infected with WNV and cDNA microarray analysis was conducted at days 1 and 3 after infection. While WNV infection did not induce any change in the expression of MMP family genes in WNV-infected HBMVE cells, significant up-regulation of MMP-1, -3 and -9 expression and activities were observed in HBCA cells at day 3 and 4 after infection. Confluent naïve HBMVE cells incubated for 6 hrs with the supernatant media from day 3 WNV-infected HBCA cells demonstrated distinct loss of TJP such as ZO-1 and claudin-1 immunostaining as compared to HBMVE cells incubated with supernatant from mock-infected HBCA cells. Furthermore, this effect was reversed in the presence of GM6001, a broad spectrum MMP inhibitor. In addition, incubation of the *in vitro* BBB model with the supernatant from WNV-infected HBCA cells compromised the integrity of BBB model, thereby implying the role of astrocytes derived MMPs in BBB opening. Overall our results demonstrate that WNV-infected astrocytes are one of the potential sources of multiple MMPs in brain which play critical role in WNV pathogenesis. Degradation of TJP by WNV-induced MMPs might lead to the opening of the BBB, ultimately allowing unrestricted entry activated immune cells as `Trojan horse' into the CNS, thereby contributing to neuropathogenesis. Because of the unavailability of WNV vaccines for humans, the prospect of using MMP inhibitors to improve disease pathology may have therapeutic relevance.

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CHARACTERIZATION OF ANTIGEN-SPECIFIC MEMORY CD8+ T CELLS FOLLOWING LIVE-ATTENUATED CHIMERIC WEST NILE VIRUS VACCINATION

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Previous Phase I studies of a chimeric West Nile virus (WNV) vaccine containing the WNV premembrane (prM) and envelope (E) sequence inserted into the yellow fever 17D vaccine genome demonstrated robust IFN-γ and cytotoxic T cell responses to WNV E antigen at days 14 and 28 post-vaccination. In the current studies, we investigated the evolution of WNV-specific CD8+ T cell phenotype and function using MHC-peptide tetramers for an epitope which is one of the dominant targets in HLA-A*02+ individuals naturally infected with WNV. Tetramer-positive cells were detected post-immunization in all HLA-A*02+ donors tested. These

cells displayed a CD45RA-/CCR7- (effector) phenotype with high granzyme B expression during the acute phase (14-28 days post-immunization). After contraction of the acute phase response (≥ 90 days post-immunization), CD45RA+ cells were predominant. Granzyme B expression was retained in 30-40% of tetramer-positive cells. An increasing proportion of epitope-specific cells expressed CD127 (IL-7Rα), a marker of T cells persisting into memory at these later time points. In approximately half of the donors, CD8+ T cells obtained 1 month post-vaccination produced IFNγ in response to *in vitro* peptide stimulation. These responses persisted to 6-12 months post-vaccination in some donors. T cell receptor sequencing showed a shift in dominant clonotypes between acute and late time points. This finding indicates that T cell clones dominating the acute response were not the same clones persisting into memory after antigen clearance. Following vaccination with this chimeric flavivirus, WNV E-specific CD8+ T cells persisted for up to one year. Antigen-specific T cells evolved from an effector phenotype in the acute phase to a long-lived memory phenotype concurrently with a shift in dominant clonotypes. The evolution of this WNV-specific T cell response is similar to that observed in successful responses to established vaccines such as yellow fever 17D.

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VACCINATION OF WILDLIFE TO CONTROL ZOONOTIC DISEASE: WEST NILE VIRUS AS A CASE STUDY

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Control of zoonotic disease by vaccinating humans is challenging and eradication is by this approach is usually not possible since humans rarely play an important role in pathogen amplification. Vaccination of wildlife offers substantial advantages including the possibility of local or broad-scale control, control efforts benefit the entire local human population (whereas human vaccination only benefits those vaccinated for zoonotic pathogens); faster and cheaper development of animal vaccines compared to human vaccines; and elimination of health risks associated with human vaccination. This approach has been successful in the local control of rabies and has been attempted in a research context, with partial success, for Lyme disease. In our study we demonstrate that vaccination of wildlife can be effective for several multi-host vector-borne pathogens by targeting species and individuals that are disproportionately responsible for pathogen amplification. We show a case study for West Nile virus and feasibility for pathogens examine other pathogens including Lyme disease and Chagas disease.

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COMPARISON OF P29, B2T AND EGHF DIAGNOSTIC PERFORMANCE (ELISA) IN PATIENTS WITH RESIDUAL CAVITIES AFTER SURGERY FOR CYSTIC ECHINOCOCCOSIS

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P29 is a metacestode-specific antigen of *E. granulosus* protoscolices that was expressed and purified as a recombinant antigen, rec P29 (Ben Nouir et al. 2009). recB2t is a truncated version of antigen B2, and has been expressed as gst-fusion protein and subsequently thrombin-digested and affinity-purifed (Hernandez-Gonzales et al., 2008). Both genes showed potential as tools for follow-up studies after surgery/treatment of cystic echinococcosis (CE) in preliminary studies. Post-surgical cavities may be difficult to diagnose even in referral centers for CE, and conventional serology results may be misleading as they may remain positive even when the cavity no longer contains viable cyst material or protoscolices, as demonstrated by diagnostic aspiration. In this preliminary, retrospective study, we compared the diagnostic performance of recP29, recB2t and (conventional) EgHf in ELISA by testing 18 patients with CE post-surgical cavities (17 in the liver and 1 in the kidney) and 1 patient with a transitional CE3b cyst in the liver. recP29 was most reliable as a means of assessing the status of residual cavity (i.e. it was negative also in cases in which recB2t and conventional EgHf were positive). Of note, recP29 was negative in the patient with the CE3b cyst.This stage is biologically active when assessed by Magnetic Resonance Spectroscopy (Hosch 2008) Our results indicate that recP29 and recB2t deserve further longitudinal studies with larger series of patients with CE.

1102

DOXYCYCLINE IMPROVES FILARIAL LYMPHOEDEMA INDEPENDENT FROM ITS ACTION ON *WOLBACHIA*

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Doxycycline, known to deplete *Wolbachia* endosymbionts from filariae, is recommended for individual drug administration in bancroftian filariasis, due to macrofilaricidal activity and its reduction of vascular endothelial growth factors and lymph vessel dilations. Lymphoedema (LE) patients benefit from reduction in disease severity up to 24 months after doxycycline, but it has remained unclear whether this is due to the antibiotic activity against *Wolbachia* or secondary bacterial infections. The purpose of this randomized, placebo-controlled trial was therefore to compare i) doxycycline with amoxicillin, which does not target *Wolbachia*; ii) the performance of either antibiotic in patients with ongoing infections (circulating filarial antigen positive and thus still harbouring *Wolbachia*) with CFA negative patients. 149 volunteers with LE stage 1-5 were randomized according to CFA status to three regimens (200mg/d doxycycline, 1000mg/d amoxicillin, or placebo for 6 weeks). All patients underwent intensive hygiene training before study onset. Reduction in frequency of acute attacks was reported after doxycycline only. 24 months after treatment significant LE progression was seen in amoxicillin and placebo patients, whereas significant improvement was monitored after doxycycline in the CFA negative and no change in the CFA positive group. Conversely, using a hygiene evaluation score, significant improvement of the hygiene was only observed in the placebo group. These results confirm, with more patients, earlier data on the beneficial effect of doxycycline against LE. They suggest however that this effect is not dependent on the actual presence of *Wolbachia*. The data also do not favour this effect being primarily due to action on secondary bacterial infection, since doxycycline effects differ from those of amoxicillin, although the bacteria promoting lymph vessel inflammation are usually susceptible to either antibiotic. Rather, the beneficial effects of doxycycline may be attributed to the recently described direct blocking effects on vessel growth and permeability.

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EFFECT OF HIGH DOSE INTRAVENOUS DEXAMETHASONE IN THE TREATMENT OF TYPHOID ENCEPHALOPATHY AMONG BANGLADESHI PATIENTS

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Typhoid Encephalopathy (TE) is one of the severe forms of enteric fever, the incidence varying between 5-30% among typhoid patients. TE usually presents with an altered state of consciousness such as disorientation, confusion, and delirium, and is associated with a high risk of fatality. The available information on the role of intravenous dexamethasone in TE is scanty and controversial. The aim of this retrospective chart analysis was to observe the outcome of the use of high dose of dexamethasone, in addition to appropriate antibiotic therapy, in TE. All the admitted patients diagnosed as typhoid encephalopathy in the Special Care Ward of the Dhaka hospital of ICDDR,B from October 2006 to September 2007 were enrolled in our analysis. Among the 23 identified patients in our chart analysis 20 patients survived and 3 died. We analyzed the overall clinical and laboratory characteristics including the use of intravenous high dose dexamethasone (3 mg/kg stat dose followed by 1mg/kg/dose 6 hourly for the next 48 hours) of these patients and compared these characteristics among the survivors and deaths. Thirteen (57%) patients were female, and the mean age of the patients was 17.5 years. All of them presented with fever with median duration of 7 days and mean temperature of 39.4° C. The median duration of diarrhea was 30 hours, and 33% had dehydrating diarrhea prior to admission. All of the isolates of *Salmonella typhi* (15) and *Salmonella paratyphi* (7) were multi drug resistant. All 20 survivors received high dose dexamethasone, and the 3 patients not receiving dexamethasone died ($p < 0.001$). Survivors less often had hypoglycaemia, as defined as random blood glucose <3 mmol/L (6% vs 100%, p=0.045). The distribution of age, Glasgow Coma Scale score, severe sepsis, WBC count, serum electrolytes and creatinine, antibiotic resistance pattern of the bacterial isolates, and the antibiotics used to treat the patients did not show any significant difference among the survivors and deaths. It is concluded that high dose dexamethasone significantly reduces the mortality among the diarrheal patients presenting with TE. Hypoglycaemia appears to be a risk factor of death in TE.

1104

THE ETIOLOGIES OF ACUTE UNDIFFERENTIATED FEBRILE ILLNESS IN AN ADULT COHORT IN BANDUNG, INDONESIA (2000-2008)

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A cohort of 3,000 factory workers in Bandung, Indonesia was followed from 2000-2004 and 2006-2008 to study dengue fever and dengue hemorrhagic fever. Volunteers experiencing fever visited the factory clinics where they received a physical examination, and had acute and convalescent blood draws. Patients who demonstrated respiratory symptoms also had nasal and throat swabs taken. The specimens collected were used for routine hematology tests and/or a series of diagnostic assays for dengue, chikungunya, typhoid and influenza. Dengue and chikungunya were confirmed by RT-PCR and/or serologic assays, typhoid by Tubex® and influenza by RT-PCR and/or virus isolation. During 66 months of observation, 1889 febrile episodes occurred. The pathogen with the highest incidence rate was influenza (4,520/100,000 population/year) followed by dengue (1,700/100,000 population/year), chikungunya (1,022/100,000 population/year) and, and typhoid (264/100,000 population/year). Further study is needed to reveal the etiologies of the remaining undiagnosed cases.

1105

THE ASSOCIATION BETWEEN KHAT CHEWING AND TOBACCO SMOKING AMONG STUDENTS OF THE COLLEGE OF HEALTH SCIENCES FOR MALES IN JAZAN, SAUDI ARABIA

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In order to design an effective tobacco control policy, it is essential to determine smoking prevalence and its associated factors including other addictive substance abuse. Khat (Catha edulis), a plant containing an amphetamine-like substance, is commonly used socially in Southwest Saudi Arabia. Studies addressing the association between tobacco smoking and khat chewing are lacking. The objective of this study was to assess the association between tobacco smoking and khat chewing among students of the College of Health Sciences for males in Jazan, Saudi Arabia. Secondary data analyis of a questionnaire based survey conducted by the same researcher in January 2007 in the College of Health Sciences in Jazan, Saudi Arabia. 571 students from all grades (77.6% of the total students) responded to a questionnaire administered by the researcher for collecting data. All the data was entered and analyzed using the *Epi-Info* program. *Chi-square* test was used as a test of statistical significance. The study revealed a 38% prevalence rate of khat chewing and a 30% prevalence rate of tobacco smoking (45.6% cigarettes, 31% hubble-bubble, and 23.4% both). Students chewing khat were more likely to smoke cigarettes ($\chi 2 = 146.7$, $p < 0.000$) and more likely to smoke hubble-bubble ($\chi 2 = 77.9$, $p < 0.000$) than those who are not chewing khat. The prevalence of cigarette smoking was 9 times higher among students who chew khat *occasionally* and 14 times higher among students who *usually* chew khat than those who do not chew khat at all. The prevalence of hubble-bubble smoking was 5 times higher among students who chew khat *occasionally* and 8 times higher among students who *usually* chew khat than those who do not chew khat at all. In conclusion, the prevalence of smoking among the students of the College of Health Sciences for males in Jazan was higher than the overall prevalence among males in the country and higher than the prevalence among health colleges students in other regions as revealed in most studies. There was an association between khat chewing and both common forms of tobacco smoking (cigarettes and hubble-bubble). The prevalence of tobacco smoking increased with the increase of khat chewing rate. Efforts of combating tobacco smoking and khat chewing should go side by side because of the association between the two habits.

1106

OUTBREAKS OF PUFFER FISH INTOXICATION FOLLOWING CONSUMPTION OF MARINE PUFFER FISH IN BANGLADESH, 2008

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Between April-June 2008 we investigated three outbreaks of marine puffer fish intoxication in three districts of Bangladesh. We identified people who had consumed puffer fish and collected information regarding illness history, local availability of the fish, knowledge about toxicity, and previous experience with consuming the fish. The team also visited Cox's Bazaar to explore supply and trade of marine puffers in one of the coastal areas of the country. We identified 95 people who had consumed the fish; 63 (66%) of them developed symptoms and 12 died. Among the 63 case-patients, 51% of the respondents were male with mean age being 29 years. The duration between consumption of the fish and illness onset was between 1-3 hours for 46% of the cases and less than 30 minutes for 22% of the cases. Tingling sensation in the body (91%), perioral numbness (68%), dizziness (64%) and weakness in the limbs (60%) were some of the common symptoms. All three outbreaks were caused by

consumption of large size (0.3-1.5 kgs) marine puffer fish. These large size marine puffers were unknown to community residents and had newly arrived in the local markets on the days the outbreaks occurred. The outbreak communities also did not have any previous experience with puffer toxicity. Ocean fishermen based in Cox's bazaar reported that they find 4/5 types of puffer fish in the sea and each weigh between 200 gms- 2 or 3 kgs. They consider puffer fish to be less valuable, since local people do not eat the fish as they know it's poisonous. Occasionally some local fishermen distribute the fresh fish to non-coastal parts of the country where people are unfamiliar with the larger variety of the fish and its toxicity in the hope of making some quick profit. In conclusion, lack of knowledge about puffer toxicity and familiarity with marine puffer fish contributed to the outbreaks. Efforts to keep the fish from being sold in the local markets and dissemination of messages about potential fatal outcomes of consuming the fish can help prevent future outbreaks.

1107

ETHICAL IMPLICATIONS OF INFORMED CONSENT IN EMERGENT CLINICAL SITUATIONS IN A "BUSH HOSPITAL" IN MALI

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Nine hours northeast of the capital city Bamako, Mali, the Dogon population has cultivated a rich and distinct culture in spite of the severe poverty and high illiteracy rates afflicting many of the inhabitants. Because of the intense seasonal transmission of malaria in this Sahelian region, the Bandiagara Health Center is an ideal field site in which to conduct a severe malaria study. We obtained Institutional Review Board (IRB) approval and initiated a genomics case-control study in September 2008 with World Health Organization-defined severe malaria cases in children less than 5 years of age that presented to the Bandiagara Health Center. As clinician-researchers, we encountered ethical questions in conducting a study where patients often presented in clinically critical conditions to a rural hospital. The study protocol dictated that blood be drawn prior to drug administration, creating a time-sensitive situation in which a parent had to understand and then consent to partake in the study. Informed consent is the cornerstone of ethically sound medical research involving human subjects, particularly in a vulnerable pediatric population. In addition to cultural, language and educational barriers, the administration of informed consent to a guardian whose child is in a clinically emergent situation is difficult. Despite the administration of an ethically sound IRB-approved protocol, questions of inducement and vulnerability may arise, and the decision-making capacity of a parent under emotional duress must be considered. Here, we explore potential gaps between the perspectives of research ethics committees and field realities, as well as the duality of principles to which we were held as both physicians and researchers in clinically emergent situations. The experience described will serve as direct feedback to IRB members, in both Mali and developed countries like the United States, and to tropical disease investigators who are involved in clinical field research with poor communities "in the bush".

1108

A GENE LINKED TO A NEW ANTIMALARIAL DRUG RESISTANCE MECHANISM: REDUCED UPTAKE BY INFECTED ERYTHROCYTES

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Human erythrocytes infected with *P. falciparum* have increased permeability to diverse organic and inorganic solutes, all attributable to an unusual ion channel known as the plasmodial surface anion channel (PSAC). Recently, two separate parasite mutants having altered PSAC activity were identified after prolonged selection with blasticidin S or leupeptin, charged organic solutes that require uptake via PSAC to access their intracellular parasite targets. Here, we used these mutants in genome-wide expression microarrays to examine possible molecular mechanisms of drug resistance. A single gene having undetectable expression led to identification of a chromosomal deletion event invariably present in leupeptin resistant parasites with altered PSAC activity. Although this locus was intact in blasticidin S resistant parasites, it was lost upon subsequent selection with leupeptin; thus, deletion of one or more genes in this locus is required for acquisition of leupeptin resistance via an altered PSAC. We then used the *piggyBac* transposition system to individually complement each of the 6 genes in the locus into a leupeptin resistant parasite clone. Complementation with only one of the genes increased infected cell leupeptin uptake, restored *in vitro* sensitivity to this toxin, and partially reversed the changes in PSAC associated with resistance. Disruption of this gene in wild-type parasites using double homologous recombination did not by itself confer leupeptin resistance. This gene encodes a heretofore uncharacterized protein, whose loss is necessary but not sufficient for acquisition of leupeptin resistance. This protein's localization and how it contributes to PSAC activity will be presented. Resistance to water-soluble antimalarial drugs may be acquired via changes in PSAC that yield reduced passive uptake across the erythrocyte membrane.

1109

CYTOKINE GENE SNPS ARE ASSOCIATED WITH SEVERE MALARIA IN VIETNAM

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Although the incidence has decreased in the last decade, malaria still remains a serious public health problem in Vietnam. As part of the MalariaGEN consortium we are undertaking studies to identify genes associated with protection from severe malaria in the Vietnamese. MalariaGEN is a global research network which utilizes a genetics approach to identify new mechanisms of protective immunity against malaria which may lead to novel vaccine development. OUCRU Vietnam is a MalariaGEN partner site and is contributing to two consortium projects. MalariaGEN has recruited ~13,000 cases of severe malaria and ~16,000 population controls for genome wide association studies (GWA) and replication studies. OUCRU Vietnam has contributed DNA samples from >1000 severe malaria cases and >2500 controls. Severe malaria patients were recruited between 1991-2008 and the cases and controls are predominantly of the Vietnamese Kinh ethnicity. 72 SNPs were genotyped by Sequenom, as part of the MalariaGEN sample QC process. Sixty-eight SNPs in 41 malarial candidate genes and 4 SNPs in the AMELX gene (gender confirmation) where genotyped in 942 cases and 2520 controls of the Vietnamese Kinh ethnicity. Eight SNPs were monomorphic, 16 SNPs were out of HWE in the controls ($P<0.05$) and 3 SNPs were associated with severe malaria: IL17RE (rs708567; OR 1.24, 95%CI 1.05-1.47, $P=0.013$), IL13:hIL-13_46457 (rs2054; OR 1.13, 95%CI 1.01-1.27, $P=0.029$) and TNF:hTNFa-1031(rs1799964; OR 1.16, 95%CI 1.02-1.31, $P=0.026$). An additional SNP was marginally associated with severe malaria: IL1A (rs17411697; OR 0.85, 95% CI 0.67-1.07, $P=0.0116$). All of the SNPs associated with severe malaria in this study are within or near cytokine genes. As severe malaria is a complex disease it is expected that multiple genes will each contribute a modest effect resulting in the overall disease risk. GWA studies are therefore a crucial step in determining all known and unknown genes involved, which may lead to the identification of new mechanisms of protective immunity against malaria.

1110

MEK/ERK SIGNALING AND REACTIVE OXYGEN SPECIES REGULATE THE MOSQUITO ANTI-MALARIAL IMMUNE RESPONSE

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Malaria is caused by infection with intraerythrocytic protozoa in the genus *Plasmodium* that are transmitted by *Anopheles* mosquitoes. In *Anopheles stephensi*, inducible nitric oxide synthase (NOS) expression regulates malaria parasite infection through the synthesis of inflammatory levels of nitric oxide and reactive intermediates. Here, we demonstrate that activation of mosquito *NOS* expression is controlled by MEK/ERK signaling pathway and reactive oxygen species production that serve as signaling molecules in mosquito cells. Specifically, in mosquito cells *in vitro* and in the midgut epithelium *in vivo*, MEK/ERK signaling negatively regulates the TGF-β1 dependent anti-malarial response. At the highest treatment dose of TGF-β1, inhibition of ERK phosphorylation increased TGF-β1-induced *NOS* expression, suggesting that increasing levels of ERK activation function to negatively feedback on induced *NOS* expression. Further, the reduction in *P. falciparum* infection levels by TGF-β1 was ERK-dependent: a reduction in ERK activation significantly enhanced TGF-β1-mediated control of parasite development. The activation of MEK/ERK signaling and *NOS* gene expression are regulated by reactive oxygen species and anti-oxidant enzymes. In particular, hydrogen peroxide dose-dependently induces MEK/ERK phosphorylation and *NOS* gene expression and these effects are reduced by catalase pretreatment. Taken together, our data demonstrate that conserved MAPK signaling and reactive oxygen species regulate mosquito cell signaling and innate immunity against malaria parasite development.

1111

SPATIAL REPOSITIONING OF GENE LOCI IN THE INTERPHASE NUCLEI OF BGE CELLS CO-CULTURED WITH *SCHISTOSOMA MANSONI* PARASITES

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The genome of mammals is highly organized when packed into interphase cell nuclei. Individual chromosomes are located in their own nuclear areas with minimal intermingling, termed chromosome territories. These chromosome territories are non-randomly positioned within cell nuclei, as are gene loci. We have found that snails are no exception and organise their genome in chromosome territories in interphase nuclei and exhibit specific nuclear radial locations for gene loci. The nuclear interior is associated with active gene expression whereas the nuclear periphery is a repressive environment with respect to transcription. Indeed, gene loci nuclear positioning has been correlated with gene expression regulation in a number of different animal species. Bge cells are a cell-line derived from an embryonic Biomphalaria glabrata snail in the 1970s. The cells can be infected with parasite *in vitro* and so provide a good model system in which to assess cellular and genomic responses to schistosoma infection. We have found that gene loci that are up regulated after infection have become repositioned in the nuclei of the infected cells, into a significantly more interior location, whereas a control gene is relocated more to the nuclear periphery. This leads us to postulate that upon parasite infection there is a major reorganization of the host genome that may be involved in controlling host gene expression. In support of this we have observed measurable and significant differences in global chromatin/histone methylation soon after parasitic infection of whole snails, as revealed by specific antibodies. The mechanism through which genome behaviour and dynamics are controlled and respond to parasitic infection could be a target to control infection.

1112

A LATERALLY TRANSFERRED BACTERIAL FERROCHELATASE GENE IS FUNCTIONAL IN FILARIAL PARASITES

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A peculiar feature of the phylum Nematoda is the apparent lack of the heme biosynthetic pathway and a metabolic dependency for extraneous heme or, in the case of most filarial nematodes, possible provision by the obligate endosymbiont, *Wolbachia*. Further analyses reveal that filarial nematodes are unusual in possessing a ferrochelatase (FC), the terminal step of heme biosynthesis, as a consequence of a lateral gene transfer (LGT) event. This enzyme catalyzes the addition of ferrous iron to the protoporphyrin ring. Genomic sequencing of the filarial nematode *Brugia malayi* revealed the presence of an open reading frame encoding a putative FC. The full-length FC transcript sequences from *B. malayi*, *Onchocerca volvulus* and the *Wolbachia*-free *Acanthocheilonema viteae,* acquired by PCR/RT-PCR/RACE analyses, provide predicted protein sequences that seem to be derived from α-proteobacterial Rhizobiales, not of *Wolbachia* origin, as inferred from phylogenetic analysis. *B. malayi* FC (BmFC) contains 9 exons spanning a ~4 kb genomic region, with the first exon encoding a 36 aa N-terminal pre-sequence, which is not present in bacterial FC. This pre-sequence contains a putative mitochondrion-targeting peptide (MTP), as predicted by TargetP. The bacterial signature residues required for catalysis are strictly conserved and complementation tests in an *Escherichia coli* mutant confirm that the filarial enzymes are functional. The N-terminal pre-sequence is not required for function. Heterologous expression in *Caenorhabditis elegans* demonstrates that the N-terminal pre-sequence is required for mitochondrial targeting, which is consistent with FC functional role in heme biosynthesis, since, in all non-plant eukaryotes possessing the heme biosynthetic pathway, FCs are exclusively mitochondrial-resident enzymes. Wild type *C. elegans* have to salvage heme for development into adulthood due to total absence of heme biosynthetic capability. However, unlike wild type worms, BmFC transgenic *C. elegans* larvae can grow into adulthood in the presence of the FC precursor, protoporphyrin, further demonstrating that filarial FCs are fully functional enzymes. It was also revealed by *in situ* hybridization experiments in *B. malayi* that the expression pattern of BmFC is different from *Wolbachia* FC. The possible implications of the existence of the last step of the heme biosynthetic pathway in filarial nematodes will be discussed.

1113

ANOPHELES CRACENS - THE VECTOR OF THE 5TH HUMAN MALARIA PARASITE, *PLASMODIUM KNOWLESI,* IN PENINSULAR MALAYSIA

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Naturally acquired cases of *Plasmodium knowlesi* in humans were thought to be extremely rare, as previously there had been only two reports of such cases, both in peninsular Malaysia. The first case was reported in 1965 in the state of Pahang, followed by a second case from Johore. *P. knowlesi* is prevalent among crab-eating macaques, *Macaca fascicularis*, pig-tailed macaque *M. nemestrina*, and leaf monkey *Presbytis melalophos*. Recently, a large cluster of human infections caused by *P. knowlesi* has been identified in the Kapit Division of Sarawak, Malaysian Borneo. Since transmission of this zoonotic parasite to humans is occurring in Sarawak, it is important to study the vectors responsible for the transmission in Peninsular Malaysia, to determine the dynamics of *P. knowlesi* transmission

to humans with the intention that control strategies for this zoonotic malaria can be implemented. A 12 hours bare leg catch and monkey-baited traps were used to collect mosquitoes that were attracted to both human and monkeys at two sites in Kuala Lipis, Pahang. All mosquitoes were identified and female *Anopheles* were dissected and midguts and salivary glands examined for infection. DNA were extracted and a nested PCR assay based on the *Plasmodium* sequence of the small subunit ribosomal RNA (SSUrRNA) were used to identify the species of malaria parasites found in the mosquito samples. Sequencing of the *csp* and SSUrRNA genes were carried out. A total of 1526 anophelines were collected. *An. cracens* was the predominant species collected (67.56%) and followed by *An. maculatus* (18.61%). *An. cracens* were the only species found to be positive for oocysts and sporozoites. All four isolates were identified as *P. knowlesi* using nested PCR assay. Sequence of the *csp* and SSUrRNA genes clustered with the reference *P. knowlesi* obtained from the GenBank and with those reported by others. In conclusion, *An. cracens* is simio-anthropophagic and acrodendrophilic and this makes it an efficient vector for the transmission of *P. knowlesi* to both human and monkey hosts. This study has incriminated *An. cracens* as the vector of *Plasmodium knowlesi* in Kuala Lipis, Pahang. Identification of vectors has been established and this should lead to the appropriate control strategies for elimination of malaria.

1114

REGULATION OF MALARIA POPULATION DYNAMICS IN SANTO, VANUATU

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Epidemiological models concerned with the control of malaria using interventions such as bednets and vaccines increasingly incorporate realistic aspects of malaria biology. The increasing complexity of these models limits their ability to abstract ecological processes and address questions on the regulation of population dynamics using time series data, particularly in regards to interactions between different pathogens and the regulatory role of immunity. We use a theoretical framework to test hypotheses on the importance of population level immunity and parasite abundance in regulating the population dynamics of malaria. We use qualitative loop analyses to examine the sign of the interaction between *Plasmodium falciparum* and *P. vivax* at the population level, and discuss implications of this sign for the within-host regulation of parasites. Our analyses of monthly malaria time series data from the island of Espirito Santo, Vanuatu (1983-1997) show that the dynamics of *P. falciparum* are not sensitive to *P. vivax*, whereas infections by the latter increase in response to those of the former. These results support a differential use of resources inside the hosts, a resource-consumer interaction between hosts and their immune system, and within-host regulation of parasites. Finally, our results emphasize the need to better understand factors regulating malaria dynamics before developing control strategies and call for the use of control strategies directed at the interruption of transmission, such as vector control and the use of bednets.

1115

DEVELOPMENT OF A MALARIA-REFRACTORY TRANSGENIC MOSQUITO USING AN ANTI-CHITINASE SINGLE-CHAIN ANTIBODY GENE

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Anopheline mosquitoes are obligatory vectors for malaria parasite transmission and are the targets for many disease control strategies. One approach is based on engineering parasite-refractory mosquitoes that could be used in genetic control efforts to curtail the spread of malaria. The first site of interaction between mosquito vectors and malaria parasites is the midgut. The *Plasmodium* ookinete must cross the chitin-containing peritrophic matrix (PM) of the midgut to establish an infection in the mosquito. Ookinetes secrete a chitinase that facilitates their passage through the PM. A monoclonal antibody (mAb), designated *1C3*, raised against recombinant *P. falciparum* chitinase, *PfCHT1*, neutralized the activities of the gene products of *P. falciparum* and its orthologue in *P. gallinaceum* (*PgCHT2*), and inhibited oocyst formation when incorporated into infectious blood meals. A recombinant single-chain antibody (scFv) derived from the *1C3* mAb reduced the mean intensities of infection and prevalence of oocysts in *P. falciparum*-infected *Anopheles stephensi* and *An. gambiae* and in *P. gallinaceum*-infected *Aedes aegypti*. The *1C3* scFv open reading frame was cloned into a *piggyBac* transformation vector under the control of the *An. gambiae* carboxypeptidase gene promoter, which confers midgut-specific and bloodmeal-inducible expression. This transgene construct was inserted into *An. stephensi*, and three lines, 4.1, 21.1 and 39.1, with 4, 9, and 9 copies, respectively, of the transgene, were recovered. RT-PCR analysis detected *1C3 scFv* transcripts in the midguts of female mosquitoes at 4h, 8h, 16h, 24h, 48h, and 72h post blood meal (PBM), and also in those offered only sugar. Real-time PCR showed strong induction of the *1C3* scFv transcription product with maximum accumulation at ~16h and ~24h PBM for lines 4.1/21.1 and 39.1, respectively. The effect of *1C3* scFv was analyzed by transmission-blocking experiments using membrane-feeding assays with *P. falciparum* gametocyte cultures. The mean intensity of infection of oocyst formation was reduced significantly in one of the transgenic lines compared with wild-type controls. This finding supports the conclusion that the presence of *1C3* scFv in the midgut of *An. stephensi* interferes with *P. falciparum* development and it is proposed that this will have an impact on subsequent parasite transmission.

1116

PERSPECTIVES OF PEOPLE IN MALI, WEST AFRICA TO GENETICALLY MODIFIED MOSQUITOES FOR MALARIA CONTROL

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Malaria continues to pose a major health problem through much of the world, especially in sub-Saharan Africa. Genetically modified mosquitoes unable to transmit the malaria parasite have been proposed as part of an integrated vector control strategy. Public acceptance is essential prior to a transgenic release, particularly since mosquitoes are a vector of human disease and genetically modified organisms face strong skepticism in both developed and developing nations. We conducted public attitude surveys in rural and urban areas of Mali, West Africa to understand local perspectives to the use of transgenic mosquitoes for malaria control. Questions were asked about nature, heredity, disease, genetic alteration and acceptable conditions for a transgenic release. Many participants perceived mosquitoes to be only one of several causes of malaria. Despite this perception, there was a widespread desire to see evidence that transgenic mosquitoes could reduce malaria prevalence, preferably through the performance of a trial. Other participants preferred that mosquitoes be killed rather than modified. A set of conditions that must be satisfied for a transgenic release to be acceptable are suggested by the range of responses given by survey participants.

1117

SPATIAL PATTERNS AND DETERMINANTS OF INSECTICIDE-TREATED NET USE, MALARIA AND ANEMIA IN THREE REGIONS OF MALAWI, 2005-2008

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Insecticide-treated nets (ITNs) are known to provide inexpensive and safe protection against Plasmodium infection among children in malaria endemic areas. Since 2005, the Malaria Watch Centre in Blantyre, Malawi has conducted a yearly household-level survey to assess the extent of infection and anemia in children under 5 years of age. We sought to 1) determine the extent and spatial-temporal distribution of malaria/anemia in surveyed areas, 2) assess the use of ITNs and the effectiveness of distribution strategies, 3) ascertain the demographic and topographical factors that may impact on infection risk, and 4) pinpoint geographic areas and subpopulations for focused interventions. During each survey, houses were geo-coded, defined clinical data were collected from children aged 6 - 30months old, and mothers were interviewed to determine demographic information and use of interventions to prevent malaria. Results indicated that rural areas of high poverty and low elevation had the most infection and anemia, and exhibited the lowest individual ITN use and lowest community ITN density. Urban areas had heterogeneous levels of ITN use and density, but fairly homogenous infection rates. Individual use and density of ITNs appeared to increase over the study period and was associated with a significant drop in infection rates, although it is difficult to evaluate causality. Bayesian spatial methods were use to create a risk map that identified areas for possible targeted ITN distribution. The complex spatial and demographic patterns of ITN use and malaria-associated ill health make efficient use of limited anti-malaria resources difficult, suggesting that more rigorous analyses of spatial patterns should improve efficiency of prevention efforts.

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INSECTICIDE TREATED NETS IN MALARIA PREVENTION: DOES DISTRIBUTION MODEL MATTER?

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Angola has one of the highest rates under-five mortality rates in the world at 25%, and malaria is the main cause of mortality, accounting for 74% of deaths. Insecticide Treated Net (ITN) is proven to be an inexpensive and effective prevention tool against malaria. However, 2003 UNICEF data showed that only 10% of children under-five slept under a mosquito net the night prior to the interview and only 2% slept under an ITN. The present study aims to address whether there are differences in use and ownership of ITNs comparing three distribution models. From June to August 2008, mothers of children 0-60 months of age were interviewed using a 27-item questionnaire at three different sites in Angola. Site 1 distributed ITN freely with attendance of an education session; Site 2 produced and sold ITN at cost; Site 3 was mixed access to ITN including free and purchased distribution. 342 mothers were interviewed (Site 1 n= 89; Site 2 n= 117; Site 3 n=136). Site was a significant predictor of ownership ($p = 0.02$, Site 1 > 3 >2) and also of ITN use (adjusted OR 7.2. $p<0.001$, Site 1 vs. 2). Among participants who owned ITN, those who attached importance to ITN usage were more likely to use it ($p<0.001$). Participants who cited neighbours as their primary source of information regarding ITN importance were more likely to use the ITN than those who cited hospital staff (adjusted OR 4.3, $p<0.001$). This study suggests and supports previous findings that free distribution with education could enhance use and ownership of ITN. In comparison to the 2003 UNICEF data, the overall rate of use of ITN is much greater at these three sites (2% versus 21.3%). Our findings also encourage future distribution programs involving peer-to-peer education as neighbours as a primary source of information can be a significant motivator.

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EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT FOR CHILDREN (IPTC) COMBINED WITH TIMELY TREATMENT AT HOME FOR MALARIA CONTROL

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Prompt and effective treatment of malaria cases is a goal desired by all as we wait for the final arrival of effective and affordable vaccine. In the interim, we need to make use of the available tools to reduce malaria related risk, especially in the vulnerable groups of children under five and pregnant women. Home management and IPT have been accepted as two important components of the malaria control strategy. The objective of this study was to demonstrate the effectiveness of using community assistants to deliver IPTC combined with timely febrile malaria-related illness management at home. The study combined home-based delivery of intermittent preventive treatment for children (IPTC) aged 6-60 months and Home treatment of suspected febrile malaria-related illness within 24 hours. All children aged 6 to 60 months received home-based delivery of intermittent preventive treatment using Amodiaquine + Artesunate, delivered at home every four months (3 times in 12 months). Malaria parasite prevalence surveys were conducted before the first and after the third rounds of deliveries of IPTC to the children. Community Assistants delivered the interventions. In our study, prevalence reduced from 25%, (baseline) to 3% (evaluation), which is about 80% reduction in malaria prevalence. At baseline, 13.8% and evaluation, 2.2% of the children were febrile (axilary temperature of ≥ 37.5). In conclusion, the evaluation result indicates that IPTC given three times in a year (every four month) combined with timely treatment of febrile malaria illness at home, could reduce malaria prevalence significantly.

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WEST NILE AND ST. LOUIS ENCEPHALITIS VIRUSES: IDENTIFICATION OF GENETIC DETERMINANTS OF ALTERED AVIAN AND VECTOR INFECTION PHENOTYPES

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St. Louis encephalitis virus (SLEV) and West Nile virus (WNV) display strikingly different vector infectivity and vertebrate pathogenic phenotypes. *Culex tarsalis*, the major vector of WNV and SLEV in western North America, is capable of becoming infected with SLEV at a lower oral titer compared to WNV, but WNV infected mosquitoes transmit more efficiently. Additionally, WNV viremia profiles in both house finches and house sparrows are approximately 10,000-fold higher than titers identified in SLEV infected birds. To identify the genetic determinants of these differential phenotypes, WNV and SLEV chimeric viruses were created and replication characterized in mammalian (Vero), avian (DEF) and mosquito cells (C6/36). In all lines, WNV replicated to titers at least 1,000-fold higher than SLEV. A chimera (SLEV-prME/WNV) consisting of SLEV structural genes (prM-E) and WNV nonstructural genes displayed similar growth kinetics and plaque morphology as the parental WNV in Vero and DEF cells, implicating the role of the nonstructural proteins for increased growth rate and cytopathogenic potential in vertebrate cells. In contrast, the reciprocal chimera, WNV-prME/SLEV, exhibited slow plaque formation and low growth phenotypes in vertebrate cells. In contrast, SLE-prME/WNV infected C6/36 cells generated titers comparable to SLEV while the WNV-prME/SLEV chimeric virus exhibited a similar phenotype to WNV, indicating the potential role of flaviviral structural genes for modulation of mosquito infectivity. These results as well as *in vivo* mosquito and avian virulence testing indicate the potential importance of SLEV structural determinants for the maintenance of low oral infection thresholds in *Culex* spp. and nonstructural determinants of WNV encoding elevated

replication phenotype in birds. These virological differences significantly influence the disparate epidemiological profiles of these agents in North America and provide evidence of contrasting evolutionary maintenance strategies of these closely related viruses.

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NORTH AMERICAN BIRDS AS POTENTIAL AMPLIFYING HOSTS OF JAPANESE ENCEPHALITIS VIRUS

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Japanese encephalitis virus (JEV) is a mosquito-borne virus with birds and pigs believed to be major amplifying hosts. The current distribution of JEV, which can cause severe neurologic disease in humans, includes portions of Asia, India and Russia, with recent emergence in Indonesia, Papua New Guinea, Australia and Pakistan. Because of the possibility of further intercontinental spread of JEV, we assessed the ability of several common species of North American birds to amplify different JEV strains in blood, as well as oral and cloacal shedding, following experimental inoculation. We also examined the protective effect of pre-existing antibodies to West Nile virus (WNV) against JEV infection. The magnitude and duration of viremia and shedding varied according to bird species and inoculum strain. In general, viremia titers were higher among birds inoculated with a Vietnamese (VN) versus Indian strain. Crows, pheasants, and chickens had minimal to no viremia following inoculation with either strain, while oral and cloacal shedding was rare in all species. Some passerine (e.g., grackles, blackbirds, finches, sparrows, starlings) and non-passerine species (e.g., pigeons, mallards) had moderate (~$10^{3.0\text{-}5.0}$ PFU/ml serum) transient viremia following inoculation with JEV VN. Most birds seroconverted by 14 days post-inoculation, with the exception of crows and some pheasants. No birds had JEV-associated morbidity, and anti-WNV antibodies in blackbirds and sparrows protected against JEV viremia. Similar to WNV, susceptibility appears to vary both among bird species and individuals. Various North American passerine birds may be competent hosts of JEV, such as the house finch (*Carpodacus mexicanus*) and common grackle (*Quiscalus quiscula*). Unlike with WNV, crows appear to be resistant to JEV infection, and in general, birds may not be susceptible to JEV-associated morbidity and mortality. Transmission of JEV may be dampened in areas with relatively high avian WNV seroprevalence. However, as with WNV, JEV infection outcome may vary by strain, and unexpected patterns could emerge should JEV arrive to the New World.

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ENTOMOLOGICAL SURVEILLANCE FOR VIRUSES IN THE YUCATAN PENINSULA OF MEXICO, JANUARY TO DECEMBER 2008

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As part of a comprehensive surveillance program for arboviruses in the Yucatan Peninsula of Mexico, 187,206 mosquitoes from 23 species were collected in the states of Yucatan and Quintana Roo from January to December 2008. Eighteen mosquito pools caused cytopathic effect in Vero cells. All consist of female *Ochlerotatus taeniorhynchus* collected in Celestun in December. RT-PCR and nucleotide sequencing is in progress to determine the identities of these viruses. Nine isolates were positive in our initial RT-PCRs performed with orthobunyavirus-specific primers. A subset of mosquito homogenates was further tested by RT-PCR using flavivirus-specific primers. This analysis was performed using at least 50 pools of *Culex quinquefasciatus* from each month. Flavivirus RNA was present in mosquitoes throughout the year. The monthly flavivirus minimum infection rates, expressed as the number of positive mosquito pools per 1,000 mosquitoes tested, ranged from 4.3 to 16.6. Approximately one-third of the RT-PCR products were sequenced and all correspond to the insect-specific virus, Culex flavivirus (CxFV). To determine the tissue tropism of CxFV in *Culex* spp. mosquitoes, female *Cx. pipiens* were infected with CxFV by intrathoracic needle inoculation and held for 7 days then selected tissues (ovaries, midguts, salivary glands and fatbodies) were removed and assayed by RT-PCR. CxFV RNA was detected in the ovaries, midguts and salivary glands but not the fatbodies of mosquitoes inoculated with CxFV isolates from Mexico and Iowa.

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YFV-INDUCED CYTOKINE EXPRESSION IN PRIMARY HUMAN MACROPHAGES

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According to the World Health Organization an estimated 200,000 people a year are infected with the mosquito-borne *Flavivirus*, yellow fever virus (YFV). YFV causes clinical illness ranging from febrile to fatal hemorrhage for which there are no therapeutics available to treat disease. Reported data for patients infected with Asibi (wild type strain) or 17-D vaccinees have indicated that certain pro-inflammatory cytokines (such as IL-6) have increased expression; as much as 21-30% difference in plasma concentrations between day 0 and 7. However, data is limited and, especially for wild-type or vaccine-adverse event patients, there is typically only one sample tested after the onset of clinical disease. Macrophages (MΦs) play a critical role in innate and adaptive immune responses. Both 17-D and Asibi viruses are capable of replicating in MΦs, but no one has yet to identify the cytokine responses resulting from these infections. Understanding the cytokine response is important for elucidating mechanisms of early pathogenesis. The aim of this study was to examine cytokine production from Asibi and 17-D infected primary human MΦs derived from donor PBMCs. Results of this study identified several pro-inflammatory cytokines that were significantly different in their production between 17-D or Asibi infected cells. Donor to donor variances were noticed, but there were consistent trends of up-regulating IL-6, IL-1β, TNF-α, RANTES, MCP-1, MIP-1α, and IFN-γ. 17-D infected MΦs typically responded before those infected with Asibi, but the response was much greater from those infected with the latter. These data indicate that 17-D infected MΦs respond early during infection with pro-inflammatory cytokines inducing a Th1 response; however MΦs infected with Asibi virus under-go an infection/response time-lag. Asibi-infected MΦs, when they respond do so with a very robust pro-inflammatory response that may exacerbate the un-controlled pro-inflammatory responses previously seen from infected hepatocytes and endothelial cells leading to a systemic inflammatory response.

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HUMANIZED ANTI-YELLOW FEVER VIRUS MURINE MONOCLONAL ANTIBODY PROTECTS AG129 MICE FROM PERIPHERAL VIRUS CHALLENGE

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Yellow fever virus (YFV), a member of the genus Flavivirus, is a mosquito-borne biosafety level (BSL) 3+ virus found in tropical regions of Africa and South America and causes severe hepatic disease and death in humans. Despite the availability of an effective vaccine (YF17D), YFV is still responsible for an estimated 200,000 YF cases and 30,000 YF deaths annually. There are currently no other prophylactic or therapeutic strategies approved for use in human YF infections. Furthermore, use of the YF17D vaccine is now being re-evaluated due to increasing reports of severe adverse events following vaccination. Previous studies have shown

virus-neutralizing murine monoclonal antibodies (MAbs) to be effective at protecting mice from flaviviral infection, but they are unsuitable for treatment of human disease due to the human anti-murine antibody (HAMA) response. To overcome the HAMA response, the humanization of murine MAbs has become increasingly more common. It is for this reason that we have humanized a YFV-reactive murine MAb, 2C9, that has previously been shown to protect mice from lethal YFV infection. The humanized 2C9 IgG was constructed by fusion of the murine 2C9 IgG variable regions with the constant regions of human IgG. We have previously reported that humanized 2C9 IgG is able to react with YF17D virus in an enzyme-linked immunosorbent assay designed to detect YFV-reactive human IgG. We have now demonstrated that the humanized 2C9 IgG possesses *in vitro* neutralizing activity comparable to that of the parent murine 2C9 IgG when assayed by plaque-reduction neutralization testing in Vero cell culture. In addition, humanized 2C9 IgG administered prophylactically 24 hours prior to infection, protected AG129 mice from peripheral YF17D challenge, which is uniformly lethal in these interferon receptor-deficient mice.

1125

PRE-EXISTING IMMUNITY TO RELATED FLAVIVIRUSES PROTECTS AGAINST INFECTION WITH JAPANESE ENCEPHALITIS VIRUS IN HAMSTERS

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Japanese encephalitis virus (JEV) is a mosquito-borne virus in the family Flaviviridae. JEV is endemic in Southeast Asia and the surrounding Pacific Islands and most recently emerged in northern Australia. The primary reservoirs of JEV are ardeid birds and domestic pigs, but spillover into humans and horses can cause severe encephalitic disease. JEV is closely related to West Nile virus (WNV) and St. Louis encephalitis virus (SLEV), two flaviviruses that are found in the U.S. Because of the threat of introduction of JEV to the U.S, it is useful to determine whether or not prior infection with WNV or SLEV can confer any immunity against JEV. To investigate this in a small animal model, groups of juvenile male Golden hamsters were inoculated once with one of the following: WNV, SLEV, 17D yellow fever virus, a chimeric yellow fever-WNV vaccine, a recombinant canarypox WNV vaccine, or Sindbis virus as a non-flavivirus control. An uninoculated group served as non-immune controls. After 28 days, all hamsters were inoculated with JEV. Hamsters were bled daily for five days and euthanized 14 days post-inoculation. Virus isolation was performed using plaque assays and antibody titers determined using plaque reduction neutralization assays, both on Vero cells. None of the hamsters infected with WNV or SLEV, or immunized with the yellow fever-WNV chimera vaccine developed detectable viremia. Six of 9 animals immunized with the canarypox-WNV vaccine, and 5 of 6 animals previously infected with 17D yellow fever or Sindbis virus became viremic. In the control group 10 of 12 hamsters developed viremia. We then examined the potential for immune serum from hamsters previously infected with JEV or WNV to protect against JEV when administered 24 hours prior to infection. Six of 6 hamsters given JEV antiserum and 3 of 6 hamsters given WNV antiserum were protected from subsequent JEV infection. These results suggest that in the event of its introduction to North American, pre-existing immunity to WNV or SLEV could mitigate the impact of JEV on susceptible hosts.

1126

LONG-TERM IMMUNITY FOLLOWING VACCINATION WITH THE INACTIVATED JAPANESE ENCEPHALITIS VACCINE IXIARO®, IC51, AND IMMUNE RESPONSE TO A BOOSTER DOSE

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Intercell's recently approved Vero cell-derived, inactivated Japanese Encephalitis vaccine IXIARO (JESPECT® in Australia, IC51) has been proven immunogenic and safe in a 0/ 28 Day schedule in adults. Protective NT (neutralizing antibody) titers are achieved in 98% of vaccinees after full primary immunization. Antibody levels decline with time. The objective of this study was to investigate the long term persistence of NTs against IXIARO and to assess safety and immunogenicity of a booster dose administered to subjects without protective NT titers. In this open-label phase III study, 349 subjects who had received different schedules of IXIARO in a preceding randomized trial were followed for 24 months after the first immunization. NTs were assessed by PRNT at month 6, 12 and 24. Subjects who tested negative for NTs at month 6 or 12 received a booster dose on month 11 or month 23 respectively, and NTs were assessed 4 weeks after the booster dose. A PRNT50 ≥ 1:10 was used as cut-off for seroprotection and seroconversion. Systemic and local tolerability of the booster dose were solicited. After full primary immunization (IXIARO on Day 0/28), seroprotection rates (SPR) were 83% (96/116), 58% (67/116) and 48% (56/116) on Months 6, 12 and 24 respectively. Booster doses in subjects whose NT titer had dropped below detection led to 100% seroconversion when given on Month 11 (N=17) or Month 23 (N=24) after primary immunization. A single 6mcg dose led to a SPR of 9% (10/117) at month 6. However, a booster at Month 11 led to seroconversion in 99% (95/96) of boosted subjects in this group. During 7 days after a booster, 21.8% and 32.6% of subjects (at months 11 and 23 respectively) reported solicited local reactions, and 17.1% and 27.9% solicited systemic adverse events. In conclusion, booster doses should be considered from 12 months after primary immunization on prior to re-exposure. Booster doses yielded >99% seroconversion rates, regardless of time point. Subjects with incomplete primary immunization can complete their schedule within at least 11 months.

1127

TRANSFORMATION OF SCHISTOSOME EGGS WITH REPORTER TRANSGENES AND MURINE LEUKEMIA VIRUS

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Transgenesis of schistosomes offers a means to elucidate gene function and expression. The egg represents an attractive developmental stage of the schistosome because of (1) ease of access to this developmental stage and its maintenance *in vitro*, (2) high ratio of germ to somatic cells, (3) miracidia that hatch from eggs can be employed to infect snails and propagate the life cycle, and (4), from the clinical perspective, the egg represents the major source of pathology in human schistosomiasis. To determine whether transgenes or other macromolecules could be introduced into intact eggs of *Schistosoma mansoni*, the eggs were incubated in Cy3-labeled siRNA or mRNA encoding firefly luciferase by square wave electroporation (1 x 125 V, 20 ms, 4 mm gap cuvettes) or soaking. Fluorescence microscopy revealed Cy3-siRNA in eggs and miracidia hatched from treated eggs. Luciferase activity was detected in extracts of eggs 3 hours after exposure to mRNA. Both results indicated that the reporter macromolecules had entered the eggs. In addition, schistosome eggs were exposed to Moloney murine leukemia virus virions (MLV) pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) by electroporation or by soaking. Analysis by end-point PCR of genomic

DNAs from miracidia from treated eggs revealed the presence of proviral MLV. Quantitative PCR analysis determined a copy number of ~30 for the luciferase transgene for every 100 copies of cathepsin D, a single copy gene in the genome of *S. mansoni*. Together these findings represent the first report of the utility of electroporation to introduce exogenous macromolecules or virions into the schistosome egg. They suggest that macromolecules enter the eggs through the cribriform pores known to form networks from the egg's exterior. The approaches confirmed the egg stage as a tractable target for germ line transgenesis and are of potential use to investigate novel therapeutic interventions since eggs trapped in liver and other tissues are at the epicenter of pathogenesis in human schistosomiasis.

1128

CHARACTERIZATION OF A MAJOR HOST-INTERACTIVE SCHISTOSOME TEGUMENT PROTEIN, SM29, USING PHAGE-DISPLAYED ANTIBODIES

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The schistosome tegument is the primary site of host/parasite interaction including parasite defense against immune recognition and effector action. Identification and characterization of host-interactive surface antigens selectively recognized by immune animals should lead to new insight into host/parasite interaction and could identify novel and promising vaccine antigens. In schistosomiasis rodent models, Fisher rats reject *S. mansoni* infections after about four weeks as compared to mice that are fully susceptible. The resistant rats mount a significantly more robust antibody response against the schistosome tegument than mice. We identified a panel of phage displayed antibodies from immune rats that recognize epitopes at the host-interactive surface of living schistosomes. The recombinant antibodies recognize both protein and non-protein antigens. In two cases, we identified the protein targets as *Sm*-TSP-2 and Sm29, which are among the small subset of schistosome proteins found to be exposed on the tegument by proteomic studies. Both antigens, especially Sm29, are selectively recognized by schistosome immune Fisher rats as compared to susceptible mice. This is consistent with literature reports showing that both of these proteins are also selectively recognized by a small subset of individuals in Brazil that appear potently resistant to schistosome infection as compared to the general population. We expressed recombinant Sm29 and specifically panned the schistosomiasis-immune rat scFv-display library for scFvs that recognize Sm29. Dozens of positives were characterized revealing five clearly distinct classes of rat anti-Sm29 scFv. These scFvs have been expressed in quantity as thioredoxin fusion proteins and are being used to characterize native Sm29 in terms of its localization and structure. Early results confirm that Sm29 is exposed to the host on living schistosomes and has some unusual structural features. These scFvs have excellent potential as reagents to characterize the host/parasite interface and to target schistosomes *in vivo*.

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PURINERGIC SIGNALING AND IMMUNE MODULATION AT THE SCHISTOSOME SURFACE

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Schistosomes are human parasitic flatworms that constitute an important public health problem globally. The parasites live for years, sometimes decades, in what is putatively a very hostile environment - the blood of vertebrates - yet they seem to elicit little if any protective reaction from two of the host's major defensive systems: the hemostatic system and the immune system. We hypothesize that this is because schistosome nucleotide metabolizing ecto-enzymes (NMEEs, alkaline phosphatase (SmAP), ecto-phosphodiesterase (SmPDE) and ecto-ATP-diphosphohydrolase (SmATPDase)), among a small subset of proteins expressed on the parasite surface membranes, dampen host pro-inflammatory and pro-thrombotic purinergic signaling mechanisms. In this way, these surface enzymes attenuate the host's ability to focus damaging thrombotic and immunological mediators in the parasite's vicinity (Bhardwaj and Skelly, 2009, Trends in Parasitology, In Press). In this work, we show that the expression of all 3 NMEE genes is upregulated following vertebrate host invasion and that all are located in the tegument, by immunofluorescence and immuneEM. RNAi treatment targeting each NMEE gene results in potent suppression of gene expression, as determined by quantitative real-time PCR and by western analysis. The viability of suppressed versus control parasites is similar in culture but is significantly diminished *in vivo*. Finally, we show that, unlike parasites whose SmAP and SmPDE genes are suppressed, parasites whose SmATPDase gene is suppressed are significantly impaired in their ability to catabolize the potent pro-inflammatory molecule, ATP. These data are consistent with the idea that some NMEEs provide an important immunomodulatory role for schistosomes within their hosts.

1130

RECOMBINANT EXPRESSION AND PURIFICATION OF CASPASE 9 OF *OPISTHORCHIS VIVERRINI*

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Cholangiocarcinoma (CCA) - cancer of the bile ducts - is associated with infection with the oriental liver fluke, *Opisthorchis viverrini*. Despite being only one of three eukaryotes designated as a 'group 1 carcinogen' by the International Agency of Research on Cancer (IARC), little is known about the transcriptome and genome of this enigmatic parasite. Caspase 9 is the apical caspase of the intrinsic pathway of apoptosis which is activated by the release of cytochrome *c* from the mitochondrion initiating interaction with the apoptosome. Caspase 9 activates the executioner caspases 3 and 7 in response to ionizing radiation, chemotherapeutic drugs, and developmental cues. The 372 amino acid (aa) open reading frame (ORF) of *O. viverrini* caspase 9 was cloned into the pET50(b)+ expression system(Novagen) encoding a recombinant caspase 9 fused with NusA to improve solubility, two hexa-histidine tags to assist in purification, rhinovirus 3C protease site to remove the fusion partner, and a carboxyl S-tag to assist in identification of the cleaved recombinant caspase 9. Expression of the caspase 9 protein was induced with IPTG in LB broth and Overnight Expression TB medium (Novagen) resulting in expression from different cellular compartments. The recombinant caspase was solubilized in 8 M urea and affinity purified on cobalt-based Talon resin, confirmed by Coomassie blue SDS-PAGE and western blot. Analyses using a panel of discrete buffers revealed that refolding of the recombinant fusion protein occurred in phosphate buffered saline, pH 7.4, 1 M urea, 1 mM dithiothreitol, 10% glycerol. Moreover, immunoblots probed with S protein, which recognizes a tag at the C-terminus of the fusion protein, suggested that autocatalytic processing of the affinity-purified, refolded NusA-caspase 9 occurred within one hour at 25ºC. The autocatalysis released the large (~35 kDa) and small (16 kDa) sub-units of the caspase. Our ongoing studies include investigation of diagnostic substrates and immunolocalization of *O. viverrini* caspase 9.

1131

PRAZIQUANTEL IS A SUBSTRATE OF A MULTIDRUG RESISTANCE PROTEIN (SMDR2) FROM *SCHISTOSOMA MANSONI*

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P-glycoprotein (Pgp) is a member of the ATP-binding cassette superfamily of proteins. It is an ATP-dependent efflux pump involved in transport of toxins and xenobiotics from cells and, when overexpressed, mediates multidrug resistance, a phenomenon initially described in mammalian tumor cells that show broad drug resistance. Pgp and other efflux transporters may be candidate targets for new anthelmintics, as they play critical roles in normal cell physiology, removal of drugs from cells, and potentially in development of drug resistance. We have used heterologous expression and fluorescence-based assays to examine the functional and pharmacological properties of SMDR2, a Pgp homolog from *Schistosoma mansoni*. Membrane vesicles from stably-transfected CHO cells expressing recombinant SMDR2 show a significant increase in rhodamine transport and ATP hydrolysis compared to those from control cells or cells tranfected with empty vector. This SMDR2-mediated transport activity is inhibited by the classic Pgp modulators (and L-type Ca_v channel blockers) verapamil and nifedipine, and is also sensitive to praziquantel (PZQ), the current drug of choice against schisotosomiasis. Transport measurements of a fluorescent analog of PZQ indicates that it is also a substrate for SMDR2. The interaction of PZQ with SMDR2 may provide an entrée into new strategies for potentiating the action of PZQ and possibly overcoming PZQ resistance.

1132

MOLECULAR CHARACTERIZATION OF WATER MOVEMENT IN SCHISTOSOMES

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Schistosomes are parasitic platyhelminths that constitute an important public health problem globally. Infection is characterized by the presence of adult worms within the vasculature of their hosts where they can reside for many years. The worms are covered by an unusual dual lipid bilayer through which they import nutrients. How the parasites import other vital molecules, like water, is not known. Recent proteomic analysis of the schistosome tegumental membranes revealed the presence of an aquaporin homolog at the host-interactive surface whose cDNA we have cloned and characterized. The cDNA encodes a predicted 304 amino acid protein (SmAQP) that is found largely in the parasite tegument by immunolocalization and is most highly expressed in the intravascular life stages. Treatment of parasites with short interfering RNAs targeting the SmAQP gene results in potent (>90%) suppression. These suppressed parasites, when placed in hypotonic medium resist swelling, unlike their control counterparts which rapidly double in volume. Additionally, SmAQP-suppressed parasites, unlike controls, resist shrinkage when incubated in hyperosmotic solution. While suppressed parasites exhibit lower viability in culture relative to controls and exhibit a stunted appearance following prolonged suppression, they are nonetheless more resistant to killing by the drug potassium antimonyl tartrate (PAT). This is likely because SmAQP acts as a conduit for this drug, as is the case for aquaporins in other systems. These experiments reveal a heretofore unrecognized role of the schistosome tegument in controlling water and drug movement into the parasites and highlight the importance of the tegument in parasite osmoregulation and drug uptake.

1133

PROTEOMIC ANALYSES OF SCHISTOSOME EGGS IN HATCHING AND DEVELOPMENT

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Schistosome eggs are the primary agents of disease in human schistosomiasis and the transmissive stage that escapes the host to further the life cycle. In view of the importance of this stage to both host and parasite, an understanding of the molecular interactions that lead to induction of the intense immune response in escaping or entrapped eggs, and to the hatching biology of eggs that eventually escape the host, is crucial for our understanding of schistosomiasis. Here we describe results of proteomic analyses of excretory/secretory product of the eggs of Schistosoma japonicum during embryonic development and hatching, using high-throughput and sensitive shotgun proteomic techniques. The constituency of secreted products of eggs from different stages of development will be considered in the light of knowledge of development and cellular organization of the embryo, eggshell and sub-shell envelopes, gleaned from correlative microscopic and spectroscopic analyses of these structures. The data presented will present a comprehensive picture of the early development of schistosomes and the nature of host-parasite interplay in this crucial parasite stage.

1134

THE INTEGRATION OF NEGLECTED DISEASES: THREE YEARS OF EXPERIENCE IN TOGO

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In Togo, programs for malaria, lymphatic filariasis (LF), onchocerciasis and guinea worm are recognized as successful while the schistosomiasis, geohelminth and trachoma programs are facing difficulties due to lack of funding. The Ministry of Health (MoH), with assistance from Health and Development International and the Centers for Disease Control and Prevention, launched a 3-phase pilot project in 2005 which aimed to integrate community-based activities of all programs mentioned above. During the 1-year preparatory phase, program coordinators determined which activities could be integrated and guidelines and tools were developed, which were piloted and evaluated during the next 2 years. The purpose of this study was to measure the impact of integration on drug coverage and bed net use through a household cluster survey (N=2135 in 2007, N=1522 in 2008). We also evaluated sanitation conditions and health knowledge as a proxy for behavioral change by asking additional questions of a randomly selected adult (> 14 yrs) of the same household (N= 291 in '07, N=186 in '08) because sustainability of Neglected Tropical Disease (NTD) control depends on both factors. Reported drug coverage for LF was similar pre- and post-integration: 80-88% in '00-'06, and 82% - 86% in '07-'08, despite the addition of treatment for schistosomiasis. Surveyed coverage during the integrated approach was 82% (95% CI 76.2-87.9%) and 77% (95% CI 69.2-84.6%). Among adult survey respondents, drug coverage in '08 was 80% with 76% who took all the offered drugs and 82% who declared that they also took the drugs in the previous year. The main reasons for non-compliance were absenteeism (28%) and treatment not offered (25%). The surveys indicated that during the second year, there was a decrease in general and disease specific health education, with only 59% of adults informed about MDA before being offered the drugs (82% in 2007). The main sources of information were community health workers and town criers (58% and 39% in 2007,

36% and 46% in 2008). The main water sources were wells (60%) and tap water (36%). Almost one in three adults collected water from an unsafe source and less than 10% of them were treating the water. Latrine use was limited to 29% of the adults. The MoH plans to expand the approach as soon as additional funding is available, but the results show that health education and sanitation practices need special attention to guarantee sustainability of this effort.

1135

THE ROLE OF PRODUCT DEVELOPMENT PARTNERSHIPS IN R&D FOR NEGLECTED DISEASES

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Product Development Partnerships (PDPs) are playing an increasingly important role in the development of new medicines for neglected diseases of the developing world, however, there has been limited information on the funding and expenditure patterns of this important group of new players. This paper analyses funding for the 13 PDPs working on neglected disease Research and Development (R&D). It uses unpublished data from the G-FINDER project, which surveyed 2007 global public, private and philanthropic investments into R&D of products for neglected diseases. In order to contribute to a better understanding of the role and significance of PDPs, we analyse at an organisational level their funders, their funding in terms of their share of global neglected disease funding, and their 'capture rate' (i.e. how dominant they are in the field they work in). We also examine the recipients of PDPs' collective expenditure. PDPs captured 22% of 'external' R&D funding for neglected diseases, i.e. funding granted by donors to research organisations, as opposed to internal investments by donors, which cannot be competed for. There was a high concentration of PDP source funding, with the Gates Foundation providing nearly half of PDPs' combined income (48%) and four public funders (United States Agency for International Development, DFID, the Netherlands government and Irish Aid) providing 29%. On average, PDPs captured 19% of total funding granted to their neglected disease R&D area. 57% of external spending by PDPs went to private sector organisations in industrialised countries, with much of it going to contract research organisations. One-fifth went to universities and public research institutes; and less than 10% to organisations based in developing countries. Our analysis confirms the central role played by PDPs in R&D for neglected diseases. PDPs facilitate private industry involvement by reducing overall risk and by channelling philanthropic and public funds to support the industry no profit/no loss model. However, their narrow funding base highlights the need to diversify their funding sources.

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HYPERTENSION IN AN URBAN SLUM POPULATION: POTENTIAL IMPACT OF SLUM HEALTH ON THE FORMAL HEALTH SECTOR

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Developing countries account for the majority of disease burden due to hypertension and cardiovascular disease. Yet, little is known about their distribution within these countries, particularly among the urban poor and nearly one billion people living in slum communities. Already 32% of the world's population and 78% of the least developed countries' urban populations live in slums. We studied the prevalence and potential impact of hypertension and related diseases in a large cohort of residents in a slum population in Brazil. A community-based hypertension survey was conducted in 2003 for 5,649 adults 18 years or older from a slum settlement in the city of Salvador, Brazil. Hypertension was defined as elevated arterial blood pressure on two separate house visits or the use of anti-hypertensive medications. Multivariate analysis was performed to evaluate risk associations for hypertension. The overall prevalence of hypertension was 16.8% (95% CI 15.9-17.8%) for the adult population (15.9% of women [14.7-17.2%] and 18.1% of men [16.5-19.6%]). In addition to age, lack of primary school education (prevalence ratio, 1.49 [1.12-1.98]) was an independent risk factor for hypertension. Among hypertensive individuals, 69.1% were aware of their illness, but only 37.3% received medical care for their hypertension. Men were less likely than women to be aware of their illness, have received medical attention or use anti-hypertensive medications. Interpretation: Slum communities are not spared from the burden of hypertension and its costly consequences. Despite awareness of their hypertension, the majority of slum dwellers did not receive adequate care from the formal health sector. Political awareness and public health interventions that specifically target these large marginalized populations are urgently needed to prevent an impending epidemic of hypertension-related diseases.

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COMPARING THE QUALITY OF INFORMED CONSENT IN THE UNITED STATES AND MALI

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Informed consent is internationally recognized as a fundamental ethical requirement for clinical research. Some commentators believe that research participants in developing countries are less likely to understand research and thus may often fail to provide valid informed consent, yet there are no available data that provide a direct comparison of the quality of informed consent among participants in developing and developed countries. We examined questionnaires used to assess participants' understanding during the informed consent process for malaria vaccine trials conducted in the United States and in two villages in Mali, West Africa. Initial responses were tallied and total scores were analyzed by age, sex, and literacy of the consenting adult, if known, and by location. 92% of initial answers by US participants and 85% of initial answers by Malian adults and parents/guardians were correct. Independent predictors of higher scores were younger age and female sex in the US (Odds Ratio (OR) 2.1 (18-25 years old) and 2.5 (26-35 years old) both compared to over 35 years, overall p=0.02; and OR 1.7, p=0.06 respectively), and male sex in Mali (OR 3.2, p=0.02). Combining results for similar questions from both locations, location was shown to be an important variable in modeling scores (p=0.005), with higher scores in the US than in Mali. Despite the difference in scores between the US and Mali, participants at both sites were well informed, with high scores overall. These results therefore do not support concerns about a systematic lack of understanding among research participants in developing as compared to developed countries.

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SELF-REPORTED HEALTH STATUS AND WELL-BEING AMONG SMALL RIVERINE POPULATIONS IN THE PERUVIAN RAINFOREST

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Small, rural communities in the Peruvian Amazon have vulnerable living conditions and minimal healthcare access, limiting their ability to cope with disease and public health emergencies. The US Department of Defense is conducting recurring humanitarian missions in riverine communities. As part of these efforts, we performed a baseline assessment to better understand the health and self-perceived wellbeing of the region's inhabitants. A cross-sectional study was conducted in four small riverine communities in Loreto, Peru, along the Huallaga and Marañon Rivers. Each village had a basic health facility. Randomly selected samples of people 15-49 years old and mothers of children <5 years of age from each community were interviewed during household visits. Their self-reported health status, healthcare usage, and sense of wellbeing, as well as their childrens' were assessed with a structured questionnaire. We surveyed 454 mothers and 450 subjects 15-49 years old. Mothers reported that 59% of the children received the full Peruvian immunization schedule. In the two weeks before the survey, 49% of the children were reported to have had a cough, 34% had a fever, and 29% had diarrhea. Among children with those conditions, 76%, 91%, and 51% sought treatment or health advice, respectively. Within these sub-groups, 60%, 60%, and 48% respectively were seen by a physician. Mothers also reported that 56% delivered their last child in a health facility, and 61% considered their children's wellbeing as good or very good. Among women and 15-49 years old combined, 56% reported being vaccinated for hepatitis B. In the last year, 18% reported having had diarrhea, 11% dengue, 8% malaria, 4% sexually transmitted diseases, and 1% leishmaniasis, although these diagnoses are likely unspecific. During their most recent episode of disease, 38% sought treatment or advice, and 60% of them visited physicians. Finally, 49% and 51% considered their physical and emotional wellbeing as good or very good, respectively. These residents of the rural Peruvian Amazon reported high levels of perceived physical and emotional well-being, despite their frequent episodes of infectious diseases and low immunization coverage. Humanitarian assistance can help to address these needs for healthcare services.

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IS THERE AN ASSOCIATION BETWEEN INFRASTRUCTURE AND DISEASE REPORTING TIMELINESS? ASSESSMENT OF AN ELECTRONIC SURVEILLANCE SYSTEM OPERATING IN A RESOURCE-LIMITED SETTING

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Timeliness plays a key role in making rapid and accurate public health decisions in disease surveillance systems; however, little is known as to whether or not it is influenced by reporting infrastructure. Therefore, we assessed this relationship in the Alerta DISAMAR system, an electronic disease surveillance system implemented among military populations in Peru. We analyzed the 2008 database from the Alerta DISAMAR system which mandates timely reporting of disease cases by health care personnel from central and remote locations via phone and/or internet. Data from each reporting facility was collected and assessed for reporting timeliness, reporting infrastructure (i.e., availability of land lines, pay phones, and internet), and personnel capacity (i.e., number of physicians and nurses). We used a clustered binomial log analysis that considered each reporting unit and its respective number of participating weeks in the system. Results were available for 90 out of 111 (81.1%) reporting units, of which 13 (14.3%) had internet, 17 (18.7%) had access to pay phone, and 40 (43.6%) had a land line. The median number of physicians and nurses were 0 and 1, respectively. A total of 4469 measures (epidemiological weeks) were analyzed using a multivariate log binomial model using the 90 reporting units. Our analyses demonstrated a significant association between timeliness, internet availability (Prevalence Ratio [PR] = 1.11 p=0.001), the number of nurses (PR = 1.01 p<0.001), and the number of physicians (PR = 1.01 p=0.017). Timeliness was positively associated with internet availability and the presence of health care personnel, primarily nurses. Studies addressing other factors directly related to reporting tasks and period of deployment are needed to further evaluate this association.

1140

PROLONGED DIARRHEA IN A BRAZILIAN COMMUNITY BIRTH COHORT: EPIDEMIOLOGY, ETIOLOGIES, NUTRITIONAL IMPACT AND LINKS TO PERSISTENT DIARRHEA

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Prolonged diarrhea (ProD, duration 7-13 days), like persistent diarrhea (PD, duration 14 days or more), is a key contributor to the global burden of childhood diarrhea and undernutrition. In contrast to PD, the epidemiology of ProD remains poorly understood, as does its connection to PD. To elucidate the epidemiology and impact of ProD in a highly endemic setting, we analyzed data on diarrheal illnesses, enteric pathogens, and anthropometrics from a 10-year prospective cohort study of 414 children born into a Brazilian shantytown. We recorded 3,257 diarrheal episodes during 1,275 child-years of observation. ProD was twice as common as PD (12% and 5% of diarrheal episodes, respectively) and accounted for a similar number of days of diarrhea (25.2% and 24.5% of days of diarrhea, respectively). ProD attack rates peaked at 6-12 months of age, followed by a peak PD attack rate at 12-24 months. ProD was more common in infants of mothers without a primary school education (RR=1.63; 95% CI, 1.02-2.78). Furthermore, early weaning was associated with earlier onset of ProD (Spearman's *rho*,.309; *P*=0.005). Kaplan-Meier analysis revealed that children with ProD before age 1 year were more likely to develop PD in later childhood (Log Rank *P*=0.002) and nearly twice as likely to develop PD by age 2 years (29.9% vs. 15.5%). ProD illnesses were associated with significant declines in weight-for-age (*d*WAZ=-0.24), height-for-age (*d*HAZ=-0.19), and weight-for-height (*d*WHZ=-0.13) Z scores (*P*<0.01). ProD-associated etiologies included *Cryptosporidium,* Eagg *E. coli,* and *Shigella* species. In conclusion**,** ProD accounts for significant morbidity, signals acute nutritional shortfalls, and shares similar risk factors and etiologies with PD. ProD in the first year of life predicts later PD and may play an important role in PD pathogenesis. Further studies are needed to address the prevention and treatment of prolonged diarrhea in resource-limited settings, with the goal of preventing persistent diarrhea, as well as long-term growth and neurodevelopmental deficits in at-risk children.

1141

MORTALITY AMONG CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA IN RURAL WESTERN KENYA, 2008

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Diarrhea is a leading cause of childhood morbidity and mortality in sub-Saharan Africa. Data on risk factors for mortality are limited. We conducted a case-control study of moderate-to-severe diarrhea among Kenyan children <5 years old enrolled in the Global Enterics Multicenter Study. A case was defined as ≥3 loose stools in 24 hrs with ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization in a child seeking care at a sentinel health center within 7 days of illness onset. Age-, gender- and village-matched controls (without diarrhea in the past 7 days) were enrolled at home. Clinical data and stool specimens were collected at enrollment. Clinical outcome was assessed at home after 60 days. From January 28, 2008 to January 25, 2009, 618 case-control pairs were enrolled; 28 (4.5%) cases and 3 (0.5%) controls died (p<0.01). Seven (25%) cases died at a health center within 4 days after enrollment, 3 (11%) died at a health center 12-36 days after enrollment, and 18 (64%) died at home (2-52 days after enrollment). Case-fatality rates by age stratum were 4.3% (<12 months), 5.5% (12-23 months), and 4.1% (24-59 months). Pathogens associated with fatal cases include: *Salmonella* 14% (5/36), enteropathogenic *Escherichia coli* 10% (7/70), enterotoxigenic *E. coli* 9% (8/91), *Cryptosporidium* 7% (4/54), rotavirus 5% (4/81), *Shigella* 4% (2/45), enteroaggregative *E. coli* 4% (5/118), *Campylobacter* 2% (2/84), and *Giardia* <1% (1/125). Co-infection was found in 14 (50%) fatal cases; no pathogen was found in 3. In a nested analysis of 27 fatal and 555 non-fatal cases, case-children who died were more likely to have had abnormal hair (48% vs. 3%, p<0.01), wasting (63% vs. 9%, p<0.01), and flaky skin (37% vs. 2%, p<0.01) on enrollment; infants who died were less likely to be breastfed (73% vs. 97%, p<0.05). Diarrheal diseases caused by a wide variety of pathogens contribute significantly to mortality. Children at highest risk are often not breastfed, and show signs of malnutrition.Despite access to health services, most diarrheal deaths occur at home.

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OUTBREAK OF TYPHOID FEVER WITH HIGH RATE OF INTESTINAL PERFORATION, KASESE DISTRICT, UGANDA--2008-2009

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Salmonella enterica serotype Typhi causes an estimated 22 million cases of typhoid fever and 216,000 deaths worldwide annually; intestinal perforation usually occurs in 1-3%. In August 2008, the Ugandan Ministry of Health (UMOH) began investigating a suspected typhoid fever outbreak with a high rate of intestinal perforation in Kasese District. In February 2009, UMOH invited CDC to join the investigation to enhance laboratory-based surveillance for *S.* Typhi and to determine the magnitude of the outbreak. A suspect case of typhoid fever was defined as fever and abdominal pain in a person with either vomiting, diarrhea, constipation, headache, weakness, joint pain, poor response to antimalarials, or intestinal perforation. Starting March 4, 2009, suspect cases in 8 clinics and 3 hospitals provided blood and stool samples for bacterial culture. Surgical specimens were examined at CDC. From July 1, 2008 through March 19, 2009, 393 suspect cases were reported. Median age was 16 years (range <1-70 years); 149 (39%) were female. Overall, 190 (48%) were hospitalized; 145 (59%) had intestinal perforation, and at least 29 (7%) died. Before March 4, *S.* Typhi was isolated from 3 of 12 stool cultures and 0 of 17 blood cultures. After enhanced laboratory-based surveillance began, *S.* Typhi was isolated from 19 (17%) of 109 patients; 14 (22%) of 65 blood cultures and 9 (12%) of 78 stool cultures were positive. Histopathologic examination of surgical specimens from 11 patients revealed perforation of the ileum with inflammation suggestive of typhoid fever. Four specimens were reactive against immunohistochemical stains for *S.* Typhi. In conclusion, this large outbreak of typhoid fever was associated with high intestinal perforation and mortality rates, possibly due to underreporting of milder illnesses, delay in appropriate therapy, or changes in pathogen virulence. A community survey is underway to help define the burden of illness and identify risk factors for infection. *S.* Typhi isolates will undergo molecular and antibiotic susceptibility testing.

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TYPHOID FEVER OUTBREAK IN KASESE DISTRICT, UGANDA: 103 CASES WITH INTESTINAL PERFORATION

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Intestinal perforation, a severe complication of typhoid fever, typically occurs in 1-3% of patients. In August 2008, the Uganda Ministry of Health began investigating a large typhoid fever outbreak in Kasese District with a reported intestinal perforation rate >50%. We reviewed medical records of patients with intestinal perforation to describe and characterize their disease and clinical course. We searched for records of all patients with intestinal perforation and surgical dates between July 2008 and March 2009 in the three hospitals with surgical facilities in the district. Information abstracted included demographics, symptoms at presentation, management, and outcome. We identified records for 103 patients with intestinal perforation. Median age was 17 years (3-53 years); 69% were male. Median duration from illness onset to admission was 9 days (1-22 days) while median time from admission to surgery was 1 day (0-11 days). Common symptoms were fever (92%), abdominal pain (91%), vomiting (40%), diarrhea (26%) and constipation (22%). Metronidazole (85%), ceftriaxone (65%), gentamycin (53%), ciprofloxacin (47%) and chloramphenicol (27%) were mainly prescribed during hospitalization. Mortality rate was 20%. Forty-three percent of patients had more than one perforation, although number of perforations did not significantly affect outcome. The terminal ileum was the site of perforation in 80% of patients. Simple repair was conducted in 41%, resection and anastomosis in 40% and colo/ileostomy in 16% of cases. Colo/ileostomy was associated with a 3.36 (p = 0.02) higher chance of repeat surgery. HIV testing was conducted in 27 patients yielded 2 positives (7.4%). In conclusion, this review found that intestinal perforation patients waited several days for medical care and that colo/ileostomy was associated with repeat surgery. Morbidity and mortality associated with typhoid intestinal

perforation can be reduced by primary health interventions targeting prevention and early treatment, and by providing proper and timely clinical and surgical care.

1144

EVALUATION OF INTERFERON-Γ RESPONSES IN PATIENTS WITH *SALMONELLA ENTERICA* SEROVAR *TYPHI BACTEREMIA* IN DHAKA, BANGLADESH

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Salmonella enterica serovar Typhi is a human restricted intracellular pathogen and the cause of typhoid fever. During infection, cellular immune responses may be required to eradicate invading organisms. Despite this, there are minimal data on cellular immune responses in humans during infection with wild type serovar Typhi. We have previously identified Typhi antigens expressed *in vivo* during human infection. To evaluate cellular immune responses, we therefore purified a number of *in vivo* expressed Typhi proteins including StaF-putative fimbrial protein-STY0202, StbB-fimbrial chaperone-STY0372, CsgF-involved in curli production-STY1177, CsgD- putative regulatory protein-STY1179, OppA-periplasmic oligopeptide binding protein precursor-STY1304, PagC-outer membrane invasion protein-STY1878, and conserved hypothetical protein-STY2195. Using an interferon-γ ELISPOT, and acute and convalescent phase samples, we next evaluated immune responses to these antigens in individuals with documented serovar Typhi bacteremia. We also measured immune responses to a crude membrane preparation of serovar Typhi (SMP). In comparison to samples collected from uninfected Bangladeshis and North American volunteers, we detected significant interferon-γ responses to SMP, StaF, StbB, CsgF, OppA, and PagC in patients. Using a proliferation assay, we confirmed increased responses in infected individuals to SMP, StaF, and PagC. StaF is a fimbrial protein homologous to E. coli YadK, and contains a Pfam motif thought to be involved in cellular adhesion. PagC is expressed *in vivo* under the control of the virulence-associated PhoP-regulon required for intra-macrophage survival of Salmonella. Our results suggest that cellular immune responses to *in vivo*-expressed serovar Typhi antigens should be evaluated in more detail.

1145

HIGH THROUGHPUT GENE EXPRESSION PROFILING OF *SALMONELLA ENTERICA* SEROVAR PARATYPHI A IN THE BLOOD OF BACTEREMIC PATIENTS IN BANGLADESH

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Salmonella enterica serovar Paratyphi A (PTA) is a human-restricted cause of paratyphoid fever, accounting for up to 1/4 of all cases of enteric fever in Asia. Analysis of PTA infection in humans has been limited. Here we report the application of a capture and amplification technique, Selective Capture of Transcribed Sequences (SCOTS), and microarray technology, to generate a bacterial transcriptional profile directly from the blood of 3 patients bacteremic with PTA in Bangladesh. We found significant mRNA differential expression (*in vivo* versus *in vitro*) for 550, 529, and 604 PTA genes in each individual patient, respectively (approximately 14% of the ORFeome of PTA). Of these, 259 genes (6.3% of ORFs) had significant differential expression *in vivo* versus *in vitro* in at least two of the three patients, and 30 genes had significant differential expression in all three patients. We identified a number of known virulence factors including genes encoded within Salmonella Pathogenicity Island SPI-1, SPI-2, and SPI-3, as well as a number of PhoP-regulated genes which are important for intramacrophage survival (phoQ, mgtC, rpoS, and slyB). We also identified 49 genes categorized as unclassified, unknown, or hypothetical that require further analysis. Using quantitative RT-PCR, we confirmed differential gene expression for identified genes. This report is the first high-throughput comparative transcriptome profiling for any pathogen in the blood of bacteremic humans, gives insight into possible new virulence factors in PTA, and suggests that such an approach may be useful in identifying pathogen-host interactions during human infection with other pathogens recoverable in low-copy number.

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GENOMIC INSIGHTS INTO LEPTOSPIRAL PATHOGENESIS

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We report the sequencing of the recently discovered intermediate leptospire L. licerasiae, using 454 pyrosequencing technology. A 20x genome wide coverage was obtained. Data analysis was performed using reciprocal blast, determination of Open Reading Frames (ORF), Pfam families of proteins and clusters of orthologs genes (COG) categories. This intermediate genome was compared to published annotated leptospiral genomes and a draft annotation was produced based on gene orthologs. We constructed a draft genome of 4.9 Mbp in 59 contigs. This genome contains 2990 CDSs, and shares general characteristics with both pathogen and saprophytic genomes. They share similar origins of replication, number of tRNA, presence of sigma factors and have components of the same general metabolism pathways. We have found no evidence of horizontal gene transfer in this analysis. Several of the suggested potential virulence factors of pathogenic leptospires are found in L. licerasiae. Some of them, including surface expressed lipoproteins seem to have undergone changes that could possible affect their function. Comparing the genomes of pathogen, intermediate and saprophytic leptospires will allow us to discover new potential virulence related mechanisms.

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REVERSE GENETIC ANALYSIS OF ERYTHROCYTE DETERMINANTS OF *PLASMODIUM FALCIPARUM* INVASION

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The absence of a nucleus in mature erythrocytes has prevented analyses of the host contribution to host-pathogen interactions. We have established the culture of mature erythrocytes from hematopoietic stem cell precursors in vitro, and we routinely obtain between 80-95% enucleation of mature erythrocytes with normal hematological properties. These cultured erythrocytes are amenable to invasion, growth, and re-invasion of Plasmodium falciparum. The combination of lentiviral transduction with our in vitro culture system allows for the generation of genetically modified mature erythrocytes. We have employed lentiviral transduction to stably express shRNAs against glycophorin A - the receptor for the major merozoite invasion ligand EBA-175. We have successfully knocked down endogenous protein levels of glycophorin A in mature erythrocytes by 74% in multiple independent experiments. We have measured the invasion efficiencies of different P. falciparum strains/invasion ligand genetic mutants into these glycophorin A knock-down cultured

erythrocytes. We observe that strains reliant on EBA-175 as the dominant receptor-ligand interaction show decreased invasion into GlyA knock down cells compared to controls, whereas strains/mutants which invade via alternative invasion pathways not reliant on glycophorin A show no inhibition of invasion. Our approach has applications in both hematology and parasitology, allowing the functional analysis of erythrocyte determinants of Plasmodium falciparum invasion and growth.

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AN UNEXPECTED ROLE OF SIR2A IN THE LIFE CYCLE OF MALARIA PARASITES

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Malaria parasites are able to evade the host immune system through altering the surface antigens expressed primarily from subtelomeric locations (process called antigenic variation). In *Plasmodium falciparum* a widely conserved histone deacetylase, SIR2 (PfSIR2A), appears crucial in the regulation of multigene families (e.g. *var* genes) involved in antigenic variation[1,2]. A second SIR2 orthologue, SIR2B, exists in *P. falciparum*. Deletion of either *Pfsir2a* or *Pfsir2b* caused transcriptional up-regulation of distinct types of *var* genes in the early asexual blood stages[3].

SIR2A and SIR2B orthologues exist in all sequenced *Plasmodium* species, including the rodent malaria parasite *P. berghei.* We have generated *P. berghei* lines that do not express either PbSIR2A or PbSIR2B and uniquely mutants lacking both proteins. In the mouse model no gross alterations were observed in asexual blood stage growth, multiplication or virulence of all 3 mutants. Global transcriptome analysis of asexual blood stages of the double deletion mutant (*Pbsir2a-/Pbsir2b-*) exhibited dysregulation of subtelomeric gene families (*bir, Pb-fam*) implicated in the process of antigenic variation as expected. *Pbsir2b* deletion had no effect on parasite growth throughout the complete life cycle. Unexpectedly, development of parasites lacking PbSIR2A was completely blocked in the mosquito host at the ookinete-to-oocyst transition. Using transgenic parasites expressing a GFP-tagged PbSIR2A protein we found a hitherto uncharacterised non-nuclear localisation focused to the apical end of the ookinete. Moreover, light microscopy analysis of ookinetes suggests an aberrant apical complex formation. These results imply that SIR2A might play a role during invasion/traversal of the ookinete of the midgut wall. Currently we are analysing the critical role for *Pbsir2a* during ookinete formation, motility, midgut barrier traversal and early oocyst formation.

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MHC CLASS II-DEPENDENT BASOPHIL-CD4$^+$ T CELL INTERACTIONS PROMOTE TH2 CELL-DEPENDENT IMMUNITY AND INFLAMMATION

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Background/Objective: CD11c$^+$ dendritic cells (DCs) are critical antigen-presenting cells (APCs) capable of priming and promoting the differentiation of naïve CD4$^+$ T cells. However, the role of DCs in the initiation of Th2 cell differentiation following exposure to helminth parasites and allergens remains unclear. The goals of this study were to directly test the role of DCs and non-professional APCs in initiating Th2 cytokine-dependent immunity and inflammation in the gastrointestinal tract utilizing infection with the helminth *Trichuris muris*. Results: By genetic restriction of MHC class II expression to CD11c$^+$ DCs we demonstrate that, in contrast to Th1 cell responses, antigen presentation by CD11c$^+$ DCs is not sufficient to generate protective type 2 immune responses *in vivo*, suggesting additional non-DC APC interactions may be required for Th2 cell differentiation. Using IL-4/eGFP reporter mice, basophils were identified as a cell population that expanded following exposure to *Trichuris* and expressed both IL-4 message and MHC class II. Depletion of basophils resulted in impaired immunity to *Trichuris* and purified basophils promoted CD4$^+$ T cell proliferation and Th2 cell differentiation *in vitro* and *in vivo*. We also identified a role for the epithelial cell-derived cytokine thymic stromal lymphopoeitin (TSLP) in driving peripheral basophilia. Conclusions: Taken together, these data demonstrate that CD11c$^+$ DCs are not sufficient to promote Th2 cell responses *in vivo* during *Trichuris* infection and are the first report of an APC function for basophils in promoting Th2 cell responses. In addition, induction of peripheral basophilia by TSLP provides a putative link between epithelial cell activation and the initiation of type 2 immunity and inflammation. These studies provide novel targets for manipulating both hematopoeitic and non-hematopoeitic cells involved in potentiating type 2 immune responses towards restoring the balance between tolerance and protective immunity at mucosal sites.

1150

NEUTROPHIL-DERIVED CCL3 IS ESSENTIAL FOR THE RAPID RECRUITMENT OF DENDRITIC CELLS TO THE SITE OF *LEISHMANIA* INOCULATION IN RESISTANT MICE

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Neutrophils are rapidly and massively recruited to sites of infection. We describe a new role in the early recruitment of dendritic cells (DCs) in response to *Leishmania major* infection. *L. major* induced the abundant production of CCL3 by neutrophils from *L. major*-resistant (C57BL/6) but not –susceptible (BALB/c) mice. The presence of CCL3 induced chemotaxis of immature DCs, an effect markedly impaired once CCL3 was depleted from neutrophil supernatant. One day post *L. major* inoculation in the ear dermis, DCs recruitment was markedly decreased in mice depleted of neutrophils prior to infection. Pharmacological or genetic inhibition of CCL3 resulted in a significant decrease in DC recruitment at the site of parasite inoculation one day post infection, while no defect of neutrophil

migration was noticed. The decrease was corrected by the transfer of C57BL/6 neutrophils at the time of infection. The early release of CCL3 by neutrophils was shown to have an impact on the development of the immune response. Altogether, we identified an essential role for neutrophil-secreted CCL3 in the first wave of DC migration at the site of infection in *L. major* resistant but not susceptible mice, with an impact in the early development of a protective immune response.

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RELEASE OF TRAP FROM THE SPOROZOITE SURFACE IS REQUIRED FOR GLIDING MOTILITY AND INVASION OF TARGET ORGANS

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Plasmodium sporozoites move by gliding motility, a form of motility that is powered by a subpellicular motor that translocates proteins posteriorly, resulting in the forward movement of the sporozoite. TRAP, a transmembrane protein, linking the motor to the extracellular substrate, is translocated posteriorly via the force of the motor and at the posterior end of the sporozoite, it is shed from the surface via proteolytic cleavage, a process that enables the sporozoite to disengage adhesive interactions and allows for forward movement. We set out to determine the function of TRAP cleavage and the nature of the protease responsible for TRAP shedding. Pulse-chase metabolic labeling experiments showed that TRAP is cleaved in its carboxy-terminus, releasing the extracellular domain from the sporozoite surface. Processing is inhibited by a subset of serine protease inhibitors. It has been hypothesized that TRAP processing is mediated by a rhomboid protease because its transmembrane domain contains a conserved rhomboid substrate motif. To determine whether this is the case and to examine the function of TRAP shedding, we generated mutants in which the putative rhomboid substrate motif was altered. Sporozoites expressing TRAP with these mutations cleave TRAP much less efficiently and the cleaved fragment is smaller, indicating that some cleavage is occurring at another site. Our data suggested that the alternate site was located juxtamembraneously and to address this issue, we generated double mutants in which the juxtamembrane region of TRAP was deleted along with disruption of the rhomboid substrate motif. These double mutant sporozoites were completely unable to process TRAP. While the rhomboid-cleavage site mutants exhibit abnormal gliding motility and have significantly decreased infectivity for target organs, the double mutants are non-motile and non infectious in both mosquito and mammalian hosts.

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DOMINANT CD8+ T CELL RESPONSES ARE ESSENTIAL FOR OPTIMAL CONTROL OF *TRYPANOSOMA CRUZI* INFECTION

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CD8+ T cells are an essential component to adaptive immune control of *Trypanosoma cruzi* infection. C57BL/6 mice infected with Brazil strain *T. cruzi* generate large numbers of parasite-specific CD8+ T cells capable of recognizing dominant TSKB20 (ANYKFTLV) and subdominant TSKB74 (VNYDFTLV) trans-sialidase gene (TS) encoded epitopes. At the peak of acute infection, a combined 30-50% of the activated CD8+ T cell population is focused against these TS-derived epitopes. With >12,000 genes in the *T. cruzi* genome and thousands of TS genes encoding variant MHC I-restricted epitopes, it is remarkable that such focused T cell populations develop. Though this is one of the largest immunodominant T cell responses described for an infection, the majority of *T. cruzi* infected mice fail to clear the parasite and go on to develop chronic disease symptoms. To determine if these immunodominant T cell populations are necessary for resistance to infection, we epitope-tolerized mice by high-dose intravenous injections of TSKB20 or TSKB74 peptides. Tolerance induction led to epitope-specific deletion of functional CD8+ T cell populations since mice tolerized to TSKB20 developed a robust TSKB74 response and TSKB74-tolerized mice developed a normal TSKB20 response. Mice tolerized against either TSKB20 or TSKB74 exhibited an increased number of parasites and cellular infiltrates in tissues during the acute phase of infection. The increased susceptibility observed was neither attributable to induction of T cells with a regulatory phenotype nor an inability to generate effector CD8+ T cell populations. We asked if TSKB20 or TSKB74 focused responses are the main protective class I MHC-restricted antigens in Brazil strain infected C57BL/6 mice by tolerizing against both epitopes simultaneously. TSKB20/TSKB74-tolerized mice exhibited transiently increased levels of tissue parasitism though ultimately controlled parasite loads. Dual-epitope tolerized mice had increased numbers of effector CD8+ T cells compared to control treated or single-epitope tolerized mice, suggesting that protective responses develop in the absence of described immunodominant T cells. These data are consistent with the hypothesis that development of high frequency CD8+ T cell populations focused on TS-derived epitopes contributes to optimal control of acute infection, though are not required for immune resistance.

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PROFILING THE TRANSCRIPTIONAL LANDSCAPE IN *TRYPANOSOMA BRUCEI* BY MRNA NEXT-GENERATION SEQUENCING

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Transcription by RNA polymerase II in trypanosomatids is still shrouded in mystery. In these organisms, Pol II transcribes long polycistronic units and individual mRNAs are rapidly matured by coupled processing events. *Trans*-splicing adds a capped spliced leader (SL) sequence at the 5′ ends of transcripts and cleavage and polyadenylation produce poly(A) tails at the 3′ ends. To analyze the transcriptional landscape on a genome-wide scale in the parasitic protozoan *T. brucei*, we performed high-throughput mRNA sequencing (mRNA-Seq) on the Illumina Genome Analyzer platform. Our data allow the precise mapping of the positions for *trans*-splicing and polyadenylation, and reveal a widespread heterogeneity in the site selection for either processing event. Additionally, we were able to identify a large number of new genes that produce properly matured *(trans*-spliced and polyadenylated) transcripts, genes with miss-annotated translation start codons in the *T. brucei* genome database, as well as annotated genes that do not produce transcripts. Importantly, a small number of polyadenylated RNAs mapped to regions in the genome regarded as putative Pol II transcription start sites. It was recently shown that such chromosome locations are marked by specifically modified and variant histones [Siegel, T.N. *et al.* (2009) *Genes Dev 23*:1063]. We find that the transcripts mapping most proximally to these loci do not possess the SL sequence or a 5′ cap structure but bear one or more phosphates at their 5′ ends. These RNAs likely represent remnants of the primary RNA polymerase II transcripts and provide evidence that such regions in the *T. brucei* genome are likely transcription start sites.

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EXPORT OF MALARIAL VIRULENCE PROTEINS THAT REMODEL INFECTED HUMAN ERYTHROCYTES

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The survival of *Plasmodium falciparum* inside erythrocytes requires extensive remodeling of the host cell by exported parasite proteins. Erythrocyte remodeling is essential for nutrient uptake, immune evasion and disease pathology, and occurs in the absence of an established protein trafficking network in the host cell. Malaria parasites employ an endoplasmic reticulum signal sequence followed by a highly conserved pentameric motif (RxLxE/Q/D) termed the *Plasmodium* export element (PEXEL) to target hundreds of proteins beyond the encasing vacuole membrane into the host cell. The PEXEL is conserved across *Plasmodia* and present in over 300 *P. falciparum* proteins. We have made significant advances in our understanding of the how the PEXEL motif facilitates protein trafficking in human erythrocytes. Here we show that an endoplasmic reticulum-resident protease cleaves the PEXEL motif and demonstrate that maturation of cargo proteins specifically by this enzyme is critical for proteins to successfully reach the host cell. The identification of the parasite enzyme that directs the export of over 300 virulence and survival proteins provides a novel target for the design of antimalarial therapies.

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