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RESTORATION OF PYRETHROID SUSCEPTIBILITY AFTER INDOOR RESIDUAL SPRAYING WITH CARBAMATES IN SENEGAL

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Vector control has played a primary role in the dramatic decreases in malaria morbidity in African countries that have scaled up malaria control in the last decade. Mass distribution of pyrethroid-impregnated long-lasting nets (LLINs) has resulted in rapid increases in ownership and use. Indoor residual spraying (IRS), usually with pyrethroids, has been implemented sub-nationally in some countries. However, gains in malaria control are threatened by increasing pyrethroid resistance. Pyrethroids remain the only class of insecticide for LLINs, but many IRS programs have switched to carbamates and organophophates to manage insecticide resistance (IR). Senegal began implementing IRS with pyrethroids in three districts in 2007 and added three more districts in 2010. Monitoring of IRS is done annually in all IRS districts and in select non-IRS sites. By 2010, pyrethroid susceptibility among female *Anopheles gambiae* had fallen in all IRS districts, with mean susceptibility to deltamethrin, lambdacyhalothrin, and permethrin of 54%, 53%, and 34%, respectively compared to 80%, 66%, and 55% respectively, in non-IRS districts. In 2011, a carbamate was sprayed in all IRS districts except one. Post-spray IR testing demonstrated an increase in pyrethroid susceptibility in the carbamate-sprayed districts, with susceptibility to deltamethrin, lambdacyhalothrin, and permethrin of 95%, 88%, and 86% respectively. The district that continued to spray pyrethroid had susceptibilities in 2011 of 72%, 41%, and 35%, respectively, and non-IRS districts had mean susceptibilities of 81%, 73%, and 64%. While susceptibility to pyrethroids increased slightly in non-IRS districts from 2010 to 2011, it increased dramatically in districts sprayed with carbamate. Testing for kdr resistance markers and species identification is ongoing to determine if a change occurred in kdr prevalence or was due to a species replacement. If spraying with a carbamate causes an increase in pyrethroid susceptibility, this may be a strategy to restore vector susceptibility to pyrethroids, prolonging the usefulness of LLINs.

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MALARIA CHEMOPREVENTION IN HIV EXPOSED INFANTS: A RANDOMIZED CONTROLLED TRIAL OF MONTHLY DIHYDROARTEMISININ-PIPERAQUINE VERSUS MONTHLY SULFADOXINE-PYRIMETHAMINE VERSUS DAILY TRIMETHOPRIM-SULFAMETHOXAZOLE VERSUS NO THERAPY FOR THE PREVENTION OF MALARIA IN A HIGH TRANSMISSION SETTING

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Insecticide treated bednets (ITNs) are currently the only widely adopted intervention for the prevention of malaria in African children. However, the burden of malaria may remain high even in the setting of ITNs in some parts of Africa with high transmission intensity. HIV-exposed children (HIV uninfected children born to HIV infected mothers) are a growing population with the added advantage of trimethoprim-sulfamethoxazole (TS) prophylaxis which has been shown to reduce the incidence of malaria. However, after HIV-exposed children stop breastfeeding and are confirmed to remain HIV uninfected, TS prophylaxis is generally stopped. A cohort of 200 HIV exposed children aged 4-5 months were enrolled using convenience sampling in Tororo, Uganda, a rural area with perennial high transmission intensity. Children received an ITN and daily TS prophylaxis per Ugandan guidelines at enrollment and were followed for all their health care needs 7 d/wk. Approximately 6 weeks after cessation of breastfeeding, 186 children (median age 10 months) who remained HIV uninfected were randomized using an open label study design to one of four treatment arms; no therapy, monthly sulfadoxine-pyrimethamine (SP), daily TS, or monthly dihydroartemisinin-piperaquine (DP). Study drugs were self-administered at home and continued until children reached 24 months of age. The primary end point was the incidence of malaria using passive surveillance. Malaria incidence was compared using a negative binomial regression model with measures of association expressed as the protective efficacy (PE=1-incidence rate ratio) after controlling for age at randomization and the incidence of malaria prior to randomization. Prior to randomization the incidence of malaria was 1.77 episodes per person year at risk (PYAR). After randomization, the incidence of malaria increased to 6.28 episodes per PYAR among those assigned to no therapy. Monthly SP was associated with a PE of 9% (95% CI -35 to 38%, p=0.65), daily TS was associated with a PE of 49% (95% CI 23 to 66%, p=0.001), and monthly DP was associated with a PE of 69% (95% CI 53 to 80%, p<0.001). Following cessation of TS prophylaxis, the incidence of malaria was very high in those children randomized to no therapy despite the use of ITNs. Extending malaria chemoprevention with monthly SP was not effective at preventing malaria, daily TS was associated with modest protective efficacy, and monthly DP was the most effective regimen.

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EFFICACY AND SAFETY OF IVERMECTIN TO PREVENT MALARIA TRANSMISSION AFTER TREATMENT OF *PLASMODIUM FALCIPARUM* INFECTIONS WITH ARTEMETHER-LUMEFANTRINE: A DOUBLE-BLIND RANDOMIZED CLINICAL TRIAL

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Artemisinin combination therapy (ACT) rapidly clears asexual malaria parasites and developing gametocytes. However, mature gametocytes persist after ACTs and malaria transmission is not prevented. Transmission after ACTs may be reduced by a drug combination that reduces the likelihood that mosquitoes feeding on a human host survive long enough to become infectious. Ivermectin (IVM) may have mosquitocidal properties but has never been used in clinical malaria trials. 120 individuals with asymptomatic *Plasmodium falciparum* mono-infection were randomized to treatment with artemether-lumefantrine (AL) alone or in combination with one or two doses of IVM in a double-blind randomized trial. Clinical safety and lumefantrine plasma levels were determined. On days 1, 3 and 7 after initiation of treatment blood samples were obtained for membrane feeding assays using a median of 94 (interquartile range 92 - 96) *Anopheles gambiae s.s.* and 24 (IQR 23 - 25) *An. funestus* mosquitoes per participant. Mosquito survival during the 10 days post membrane feeding and mosquito infection status were determined. The AL-IVM drug combination was well tolerated. Mosquitoes experienced a 3- to 4- fold reduced survival when feeding 1 day after IVM (p<0.001 for both species) and a 1.4-fold reduced survival when feeding 3 days after IVM (p=0.007). The double dose IVM resulted improved the duration of the mosquitocidal effect and showed a modest reduction in mosquito survival until day 7. In conclusion, our results indicate a significant but short-lived effect of IVM on mosquito survival rates and support a role for IVM in preventing malaria transmission after ACTs.

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IMPACT OF CHOICE OF ANTIMALARIAL TREATMENT REGIMEN ON THE SELECTION OF DRUG RESISTANCE-MEDIATING POLYMORPHISMS IN *PLASMODIUM FALCIPARUM* IN UGANDA

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Artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) are artemisinin-based combination therapies (ACTs) used to treat *falciparum* malaria. ACTs pair potent, short-acting artemisinins with longer acting drugs that eliminate persisting parasites. However, slowly cleared partner drugs may select for reduced drug sensitivity. Polymorphisms in *Plasmodium falciparum pfcrt* and *pfmdr1* impact upon sensitivity to a number of drugs, with the same mutations that mediate resistance to chloroquine (CQ) and amodiaquine leading to increased sensitivity to other drugs, including lumefantrine and artemisinins. To investigate the selective pressures of AL (the national first-line regimen) and DP on resistance-mediating polymorphisms and to track polymorphism prevalence over time, we studied *P. falciparum* isolated from Ugandan children randomized to treatment with either AL or DP for every episode of uncomplicated *falciparum* malaria from 2008-12. Genotyping utilized a recently optimized ligase detection reaction fluorescent microsphere assay. 950 randomly selected malaria episodes distributed equally over time from each treatment arm were genotyped at 6 *loci* (K76T in *pfcrt*; N86Y, Y184F, S1034C, N1042D, and D1246Y in *pfmdr1)*; studies of additional *loci* (I876V and K1466R in *pfmrp1*) are ongoing. Over time, we found a steady and statistically significant increase in the frequency of the wild-type alleles at *pfcrt*-76, *pfmdr1*-86, and *pfmdr1*-1246 in both treatment arms, indicative of changes in parasite populations in Uganda with decreasing use of CQ and increasing use of AL. However, both the rate of increase and the final proportion of wild-type alleles were much more pronounced in the AL treatment arm in comparison to the DP arm. These results highlight both the changing molecular epidemiology of malaria in Uganda in the context of changing treatment practices and the differential impacts of different therapies on parasite resistance.

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GENETIC LINEAGES OF EMERGING SULFADOXINE-RESISTANT *PLASMODIUM FALCIPARUM* IN PREGNANCY-ASSOCIATED MALARIA IN MALAWI

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The emergence of drug resistant parasites threatens the efficacy of efforts to prevent pregnancy-associated malaria. In Malawi, *Plasmodium falciparum* parasites bearing the A437G and K540E mutations in the dihydropteroate synthase (dhps) gene ("double mutants") became fixed by the mid-2000s, concomitant with a decline in the efficacy of IPTp-SP. Using parasites from 1997-2010 collected from placental specimens, we monitored for the appearance of parasites bearing the additional A581G mutation in dhps ("triple mutants") and investigated their genetic relationships with double mutant haplotypes using microsatellite markers linked to the dhps gene. Microsatellite profiles were generated at 5 *loci* for 114 *parasitemia*s collected from placental specimens between 1997-2010: 25 wild type parasites (SAKA), 68 double mutants (SGEA), 10 triple mutants (SGEG), and 11 others. Of the major dhps haplotypes, microsatellite heterozygosity (He) was lower for mutant haplotypes SGEA (He 0.454) and SGEG (He 0.485) than wild type SAKA (He 0.798) parasites. By pairwise measures of genetic connectivity, the triple mutant SGEG haplotypes were more closely related to double mutant SGEA parasites by PhiPT (0.036; p = 0.17) and Nei's genetic distance (0.07) than to other haplotypes. Median-joining network analysis of microsatellite haplotypes demonstrated the clustering of triple mutants with haplotypes bearing double mutants. Furthermore, a clonal lineage analysis predicted a shared lineage between triple and double mutant haplotypes that predominate in Malawi. The close genetic relationships between double and triple mutant parasites suggest that the parasites in Malawi that bear the dhps triple mutant SGEG haplotype less likely migrating into Malawi from other sites in East Africa but more likely emerging from established local parasite populations. Further studies are needed to compare triple mutant SGEG haplotypes with other African sites and to characterize the impact of parasites bearing these mutations upon the efficacy of antenatal malaria control strategies.

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FALCIPARUM MALARIA IN THE GREATER MEKONG SUB-REGION: MAPPING GENE FLOW AND GENOMIC SIGNATURES OF DRUG RESISTANCE

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In response to reports of emerging drug resistance to artemisinins in Western Cambodia, genome-wide association studies have recently been

done to search for the molecular cause(s) of resistance. We now aim to complement these initial efforts by using genomics to study the migration patterns of *Plasmodium falciparum* parasites in and out of the focus of origin of *P. falciparum* artemisinin resistance in Western Cambodia. We used standard population genetics parameters as well as coalescent theory to estimate population structure, divergence, and migration. Coupled with these population and migration analyses, we investigated both the effects of drug resistance-associated positive selection on the genome to look for newly-emerged *loci* possibly selected by exposure to artemisinin. We used cross-population extended haplotype homozygosity to identify regions of the *P. falciparum* genome under positive selection surrounding the pfcrt, pfdhps, pfdhfr, and pfmdr-1 genes responsible for resistance to chloroquine, sulphadoxine, pyrimethamine, and quinolone compounds, respectively. We looked for shared characteristics of signatures surrounding these *loci* and others of unknown origin that could play a role in recently-emerged drug resistance. Using the coalescent migration program Lamarc we observed non-symmetrical migration of parasites from the resistant parasite population in Western Cambodia to points across Southeast Asia. We also found that areas of the genome associated with known drug resistance *loci* have the highest selection scores, and identified multiple additional regions under selection that had high selection scores and no previous association with drug resistance. These results will be discussed in relation to *loci* associated with artemisinin resistance phenotypes in genome-wide association studies.

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WITHIN-HOST COMPETITION OF MALARIA PARASITES IN HUMANS AND THE FITNESS COST OF DRUG RESISTANCE

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Most infections with the malaria parasite *Plasmodium falciparum* consist of multiple genetically distinct strains, probably resulting from multiple infective mosquito bites in endemic countries. Because these different strains presumably occupy the same ecological niche inside the human body, within-host competition might be expected to occur in mixed-strain infections. If such competition occurs, drug-sensitive strains may suppress transmission of drug-resistant strains, which could slow the evolution of resistance and/or accelerate its decline following the retirement of a failing drug. In order to test the hypothesis that within-host competition occurs in mixed-strain infections, we used quantitative real-time PCR to determine *parasitemia* values of chloroquine-sensitive and chloroquine-resistant *P. falciparum* in 746 blood samples from Ghana (obtained from four locations between 1999 and 2010). Total *parasitemia* did not differ between single and mixed infections (p=0.16), while the density of drug sensitive and resistant parasites was reduced in mixed infections relative to single infections (p=0.0006 and p=0.002 for chloroquine-sensitive and chloroquine-resistant parasites, respectively). These findings suggest that competition does indeed occur when multiple strains infect individual hosts. Further results suggest a fitness cost of drug resistance: first, chloroquine-resistant parasites attained lower densities than chloroquine-sensitive parasites in both single and mixed infections (p=10^-14 and p=10^-5, respectively); second, the frequency of chloroquine resistant parasites in the sampled population dropped from 78% in 2004 (the last year that chloroquine was used in Ghana) to 43% in 2010 following the cessation of drug pressure. This finding corroborates previous observations of decline in resistant alleles following changes in drug policy. In summary: these findings provide evidence that within-host competition occurs in endemic regions and have implications for our understanding of how drug-resistant parasites evolve in different transmission settings.

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THE EFFECT OF DRUG QUALITY ON THE SPREAD OF ANTIMALARIAL RESISTANCE IN *PLASMODIUM FALCIPARUM*

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Antimalarial resistance has far-reaching global health implications, threatening not only the well-being of patients but the goal of control and ultimately the elimination of malaria from endemic countries. The use of substandard treatments, along with the availability of falsified medicines, undermines this effort. Clinically, substandard treatments are increasingly recognised as an important cause of patients receiving less than the required therapeutic dose of the active ingredient(s); this can arise, either from poorly manufactured products, or falsified medicines. Administration of such subtherapeutic drug concentrations results in selective pressure on the parasite to evolve antimalarial resistance thus enhancing its propagation in a population. The aim of this research was to mathematically model the effects of drug quality on *Plasmodium falciparum* antimalarial resistance in Kenya. For the purpose of this study, poor drug quality was defined to be the use of falsified medicines and substandard treatments. A deterministic mathematical model was developed, with sensitivity analyses and validation methods used to test the model, using data from the Worldwide Antimalarial Resistance Network and the World Health Organisation. The key findings from this model will be discussed, along with the insight the model provides to the current issues of antimalarial resistance control strategies. This study is timely as the effect of drug quality on antimalarial resistance throughout communities is of utmost importance. Antimalarial resistance threatens not only the life of the patient but also the effectiveness of global malaria control programmes and regulatory control of medicines. The relationships identified contribute to the growing body of evidence of the impact that drug quality has on antimalarial resistance and will further inform stakeholders as to where resources can be best utilised.

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MUTATIONS OF *PFSERCA* GENE AFTER NINE YEARS OF ARTEMISININ COMBINATION THERAPY FOR *PLASMODIUM FALCIPARUM* MALARIA IN THE PERUVIAN AMAZON BASIN

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After the emergence of *Plasmodium falciparum* drug resistance to chloroquine and sulfadoxine-pyrimethamine, Peru was the first South American country to implement artemisinin combination therapy (ACT) based on artesunate (AS) and mefloquine (MQ) in 2001. The *P. falciparum* sarco/endoplasmic reticulum Ca 2+-ATPase (*PfSERCA*) gene was previously reported to be associated with resistance to artemisinin derivatives. The potential emergence of drug resistance requires continual monitoring

for mutations in drug resistance markers; thus, *PfSERCA* was assessed in Peru in 2006-2007 (Bacon et al 2009). In continuation of that study, we analyzed 88 samples collected more recently between 2007 and 2012 in the Peruvian Amazon Basin for 11 mutations in *PfSERCA*: L263D/K, F264L, L402V, E431K, S466N, A623E, A630S, S769N, a G codon deletion in 884, V1168I and a synonymous mutation at C1031(TCC >TGT). We determined that six alleles remained wild type (L263D/K, F264L, E431K, A623E and S769N), and S466N remained very infrequently polymorphic. However, we found substantial increases (>40%) in the frequencies of mutants L402V, V1168I and A630S ($p<0.001$), previously present but below 10%. The synonymous mutation at 1031 also increased from 14% to 30% ($p<0.01$). Finally, the frequency of the G codon deletion at 884 decreased from 77% to 22% ($p<0.001$). Eight genotypes were documented in the samples from 2006-2007, including a wild type (4%); the most frequent were the G codon deletion at 884 (69%), and the synonymous mutation at 1031(11%). In contrast, eleven genotypes were identified without any wild types in the samples from 2007-2012. The two most frequent were the 3M 402/630/1168 (n=39, 44%) and the 2M 630/1030 (n=24, 27%). Sixty percent of the current samples were identical to previously documented strains while 40% represented new genotypes. The observed changes in the *PfSERCA* genotypes suggest the circulation of different *P. falciparum* strains, perhaps in response to ACT. Therefore, the role of selective pressure by AS and MQ on these changes should be evaluated and monitored prospectively.

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CHALLENGES IN THE INTERPRETATION OF DENGUE VACCINE TRIAL RESULTS

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Several previously published hypotheses have been proposed to explain the unexpected results of the first completed clinical trial of a vaccine against dengue virus. The vaccine was effective in reducing the incidence of clinical disease caused by dengue serotypes 1, 3 and 4, but failed to reduce the incidence of dengue-2 (DENV-2). The authors of the study propose potential explanations including an antigenic mismatch between the parental strain of the DENV-2 component and currently circulating DENV-2 viruses in Thailand, an increased role for immunity to non-structural proteins in DENV-2 that this vaccine does not induce and a lack of correlation of measured neutralizing antibody and protective immunity. However, we believe that in addition to questioning the immune response elicited by the vaccine, it is important to discuss the interpretability of efficacy results for dengue vaccine trials that are based exclusively on clinical outcomes. A previously publisher study measured vaccine efficacy against clinically apparent infection. This is distinct from vaccine efficacy against infection, and potentially a very important distinction. To explore the agreement between vaccine efficacies against infection and against clinical infection --under different assumptions of the impact of prior heterologous immunity on the probability of symptomatic disease-- we developed an analytical probabilistic framework that allows these efficacies to be quantified. Our results suggest that the vaccine efficacy against clinically apparent infection often leads to large underestimates of the vaccine efficacy against infection but can also lead to overestimates depending upon the tradeoff between preventing infections and inducing immunity that can predispose individuals to a more severe outcome. Discarding or moving forward with vaccine candidates purely on the basis of its efficacy against clinical disease could lead to prematurely abandoning vaccines that have promising biological action or moving forward with an overly optimistic estimate of a vaccine's impact.

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POTENTIAL OPPORTUNITIES AND PERILS OF IMPERFECT DENGUE VACCINES

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The ideal dengue vaccine is one that has high and equal efficacy against all four serotypes. However, results of a recent Phase IIb trial indicate that the vaccine candidate furthest along in development protects against serotypes 1, 3 and 4 but not serotype 2. While partially effective vaccines may have a positive impact on morbidity and mortality, particular profiles could result in increased clinical disease due to antibody dependent enhancement. The potential population-level impacts of a partially effective vaccine have not been explored. We developed a four-serotype, age-specific compartmental dengue transmission model that takes into account cross-protection and interaction between serotypes. We calibrated the model to capture transmission dynamics in traditionally hyperendemic setting (Rayong, Thailand), using data from a recently conducted age-stratified serological survey and age-specific incidence data. We also considered scenarios where the disease has re-emerged more recently (Mexico, and Brazil). We used the model to assess the potential impact of partially effective vaccines at the population level and to estimate direct and indirect vaccine effects. Crucially, we evaluated the effects that heterogeneities in pathogenicity, transmission intensity and enhancement between serotypes may have in the presence of mass vaccination campaigns. In the majority of scenarios explored, the impact of partially effective vaccines was positive and resulted in 50% or greater reductions in the number of cases. This was true even of vaccines that we would not expect to be licensed due to poor or incomplete immune responses. Our results also show that partially effective vaccines can have significant impacts on the mean age of cases and on serotype distributions, due to reduced competition for susceptible individuals. The magnitude of direct vs. indirect vaccine effects depended on the particular scenario explored. Dengue vaccine development efforts have focused on the development of tetravalent vaccines. Our results suggest that despite the virologic and immunologic characteristics of dengue, partially effective vaccines have the potential to be important tools for dengue control. Consideration of imperfect vaccines will require careful characterization of the epidemiology of dengue in each place.

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THE LIVE ATTENUATED TETRAVALENT DENGUE CANDIDATE VACCINE TV003 IS WELL TOLERATED AND HIGHLY IMMUNOGENIC IN FLAVIVIRUS-EXPERIENCED SUBJECTS

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Dengue virus (DENV) has become the most important arbovirus worldwide with nearly 400 million infections occurring annually. To develop a live attenuated tetravalent (LATV) dengue vaccine with the most favorable safety and immunogenicity profile, the US National Institutes of Health evaluated different monovalent and tetravalent DENV candidate vaccines. Following a single subcutaneous dose of admixture TV003 in flavivirus-naïve subjects, 74% of vaccinees became seropositive to all 4 DENV serotypes and 92% became seropositive to ≥ 3 serotypes. Prior to evaluating TV003 in dengue endemic areas, its safety, replication,

and immunogenicity were evaluated in flavivirus-experienced subjects. Volunteers with a documented history of flavivirus exposure or flavivirus immunization were recruited in the Baltimore MD and Burlington VT areas. 56 subjects were enrolled in this trial; 40 subjects received TV003 and 16 received placebo. Each of the components of TV003 was given at a dose of 1,000 PFU. Six months after receipt of the first dose of vaccine, subjects were challenged with a second dose. They were followed in an identical manner after both doses. Viremia and safety labs were assessed on days 0, 3, 6, 8, 10, 12, 14, and 16 after each immunization. Specimens were collected for serological analysis on study days 0, 28, 56, 90, 150, and 180 post-immunization. Sixty percent of all vaccinees had at least one vaccine virus recovered from the blood following the first immunization; none had detectable vaccine virus in the blood following the second dose. Although the mean peak titer of two of the vaccine components was slightly higher in flavivirus-experienced subjects compared with flavivirus-naïve historical controls, there was no significant difference in the adverse event profile. Following the first immunization, the vaccine induced a tetravalent neutralizing antibody response in 85% of vaccinees and a trivalent or better response in 100% of vaccinees. Complete safety and immunogenicity data following the first immunization will be discussed as will differences noted between the responses in flavivirus-naïve (historical data) and flavivirus-experienced subjects. The safety, absence of viremia, and immunologic response of vaccinees to the challenge dose of vaccine will also be discussed.

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THE TYPE-SPECIFIC NEUTRALIZING ANTIBODY RESPONSE ELICITED BY A DENGUE VACCINE CANDIDATE IS FOCUSED ON TWO AMINO ACIDS OF THE ENVELOPE PROTEIN

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Dengue viruses are mosquito-borne flaviviruses that circulate in nature as four distinct serotypes (DENV1-4). Severe clinical manifestations of disease are predominantly associated with a secondary infection by a heterotypic DENV serotype. The increased risk of severe disease in DENV-sensitized populations significantly complicates vaccine development, as a vaccine must simultaneously confer protection against all four serotypes. Eliciting a protective tetravalent neutralizing antibody response is a major goal of ongoing vaccine development efforts. However, a recent large clinical trial of a candidate DENV vaccine revealed low protective efficacy despite eliciting a neutralizing antibody response, highlighting the need for a better understanding of the humoral immune response against DENV infection. To identify epitopes recognized by serotype-specific neutralizing antibodies elicited by monovalent DENV1 vaccination, we constructed a panel of over 50 DENV1 structural gene variants containing substitutions at surface accessible residues of the envelope (E) protein to match the corresponding DENV2 sequence. Amino acids involved in recognition by serotype-specific neutralizing antibodies were identified as DENV variants with reduced sensitivity to neutralization by DENV1 immune sera but not by cross-reactive neutralizing antibodies elicited by DENV2 vaccination. We identified two mutations that contribute significantly to type-specific recognition by polyclonal DENV1 immune sera. Longitudinal and cross-sectional analysis of sera from 24 participants of a phase I clinical study revealed a markedly reduced capacity to neutralize a DENV1 variant containing the two mutations. Sera from 77% of subjects recognized the DENV1 variant and DENV2 equivalently (<3-fold difference). These data indicate the type-specific component of the DENV1 neutralizing antibody response to vaccination is strikingly focused on just two regions of the E protein. This study provides an important step towards deconvoluting the functional complexity of DENV serology following vaccination.

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DENVAX ELICITS TYPE-SPECIFIC AND BROAD NEUTRALIZING ANTIBODY RESPONSES REACTIVE TO DIVERSE DENV ISOLATES

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An important prerequisite for successful vaccination against dengue viruses (DENV) is the induction of neutralizing antibody responses to all four serotypes. Ideally, such a response should be type-specific and have a broad cross reactive potential against genetically and geographically diverse DENV isolates from each serotype. In our studies we characterized; i) the potential of DENVax to elicit type-specific neutralizing antibody responses, and ii) the breadth of neutralizing antibody responses elicited by a tetravalent DENV vaccine based on the DENV-2 PDK-53 cDNA clone genetically engineered to express the prM and E genes of DENV-1, -3, and -4 (DENVax). Depletion of anti-DENV-2 neutralizing antibodies from DENVax immune serum had no significant impact on the neutralizing titers to the other three serotypes. We also used a panel of 24 DENVs collected the last two decades from different tropical and subtropical regions of the world to screen immune serum elicited after DENVax immunization of non-human primates or naïve individuals from a phase I clinical trial. Immune serum from both species collected after secondary immunization exhibited broad neutralizing activity across all the DENV serotypes. Taken together these data suggest that DENVax could elicit type-specific neutralizing antibody responses and be potentially effective in protecting against contemporary DENV strains circulating in endemic areas.

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A PHASE 2 AGE-DE-ESCALATION CLINICAL TRIAL OF A RECOMBINANT LIVE ATTENUATED TETRAVALENT DENGUE VACCINE (DENVAX) IN HEALTHY VOLUNTEERS FROM ENDEMIC COUNTRIES

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We have been developing a tetravalent, live attenuated dengue vaccine (DENVax) consisting of a molecularly characterized, attenuated DENV-2 strain and three chimeras in which the prM and E genes of the attenuated DENV-2 were substituted with those of DENV- 1, -3 or -4 viruses. We have previously demonstrated that tetravalent formulations of DENVax were safe and immunogenic after either subcutaneous or intradermal administration in Phase 1 clinical trials in healthy, flavivirus negative adults. We have now evaluated DENVax in a Phase 2 age-de-escalation clinical trial conducted in healthy volunteers (ages 1.5-45 years-old) in four endemic countries (Puerto Rico, Colombia, Thailand and Singapore). DENVax was administered at 0 and 3 months by subcutaneous injection. A total of 148 subjects (76M: 72F) were dosed in four sequential age cohorts

with data from each cohort reviewed by an independent Data Safety Monitoring Board before the next younger cohort was dosed. There were no related serious or severe adverse events (AEs), and no discontinuations due to vaccine-related AEs. The most common AEs included headache (18%), upper respiratory infections (15%) and pharyngitis (14%). The most common laboratory changes were decreased hemoglobin (47%, all Grade 1), decreased fibrinogen (17%), decreased WBC (14%, all Grade 1). Transient local, reactogenicity was noted in about 25% of subjects. Thus, the vaccine was well-tolerated with mostly mild and transient local or systemic reactions. Preliminary analysis reveals that DENVax induced significant neutralizing antibody responses to all four dengue viruses after one or two administrations: 98.8% of subjects were seropositive for three or more dengue viruses and 87.2% were seropositive for all four dengue viruses one month after second dose. This study highlights the safety and immunogenicity of the tetravalent DENVax vaccine in children and adults in dengue endemic countries. DENVax warrants further evaluation in Phase 2b/Phase 3 efficacy studies.

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PRECLINICAL AND CLINICAL TESTING OF A RECOMBINANT SUBUNIT VACCINE FOR DENGUE

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Dengue viruses are a major cause of morbidity and mortality throughout the tropics and subtropics. It is estimated that more than 120 countries currently have endemic dengue virus transmission. Each year there are at least 50-100 million infections, of which 2.1 million are clinically severe, resulting in more than 21,000 deaths. While a licensed dengue vaccine is not yet available, several vaccine candidates designed to protect individuals against dengue virus-induced disease are currently being evaluated in clinical trials, including a tetravalent recombinant subunit vaccine. Preclinical studies of this recombinant subunit vaccine have been conducted in non-human primates to evaluate the immunogenicity and efficacy of tetravalent formulations. Vaccine formulations have been evaluated in both dengue naïve and experienced animals. These preclinical studies have shown the capacity of the recombinant proteins to induce balanced tetravalent responses without evidence of interference. Data from these preclinical non-human primate studies will be presented. The vaccine candidate is also being tested in a Phase 1 clinical trial in healthy, flavivirus-naïve, adults. An update on clinical trial status will also be provided.

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UPDATE ON EVALUATION OF AN ATTRACTIVE LETHAL OVITRAP (ALOT) AGAINST *AEDES AEGYPTI* FOR DENGUE CONTROL IN IQUITOS, PERU

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We have developed a lethal ovitrap (Attractive Lethal OviTrap = ALOT) for dengue prevention, with concentration on identification of mosquito oviposition attractants and stimulants. Since June 2011, we have tested the ALOT in a field trial in dengue-endemic Iquitos, Peru. The study design was a prospective nonrandomized controlled trial in two cohorts of Iquitos residents from two comparable city neighborhoods each of 2500 houses selected as either intervention or control zones. Traps were placed at a density of ~3 per residence, with ~85% participation in the intervention area. Local ministry of health fumigation to control adult mosquitoes was ongoing in both areas during the study. Entomological indices were monitored in participating households at 3 month intervals, and individuals were monitored serologically, both through a longitudinal survey (at months 0, 12, 24) and through 3X weekly febrile surveillance. One year into the trial, dengue incidence as measured by febrile surveillance was 75% lower (0.26% vs. 0.97%) in the intervention area compared to the control area ($p<0.0001$). Longitudinal surveillance showed that people in the ALOT area were 25% less likely to contract symptomatic or asymptomatic DENV than those in the control area. Incidence was 12.67 vs. 17.05 in ALOT vs. Control areas, respectively ($p=0.03399$). The proportion of nulliparous females (compared to parous and/or gravid females) was significantly higher in the ALOT area, supporting the removal of older egg-laying mosquitoes from the population. This held true for both the targeted species, *Ae. aegypti* ($p<0.0001$), and for non-target *Culex* species ($p=0.0030$). Finally, if the ALOT differentially attracts females vs. males, we would expect a skewing of the sex ratio over time to show fewer females relative to males. There was a highly significant difference in sex ratio ($p<0.0001$), between the ALOT and Control sites. The ratio in the ALOT area started at 50:50 and is now at 60:40 (male to female) while the Control area has not changed from 50:50 since start of the project in June 2011.

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RIVER BOATS CONTRIBUTE TO THE SPREAD OF *AEDES AEGYPTI* MOSQUITOES IN THE PERUVIAN AMAZON

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In the Americas, as in much of the rest of the world, the dengue vector *Aedes aegypti* is predominantly found in urban areas. Its presence in rural areas is more limited, and the factors favoring its potential geographic expansion to rural and smaller urbanized settings are poorly understood. In the Peruvian Amazon, this vector has been expanding its range into rural communities over the last 5-10 years. Understanding *Ae. aegypti* geographic expansion is important for anticipating the future range expansion of dengue and other viruses transmitted by the mosquito. To examine the hypothesis that accidental transport of mosquitoes by human vehicles plays a significant role in such expansion, we measured adult and immature *Ae. aegypti* abundance in a total 97 fluvial and terrestrial vehicles departing the city of Iquitos. River transit included small public transit boats (peque-peques), medium-sized boats transporting cargo and passengers over short (avg. of 96.2 km) distances (lanchitas) and large boats transporting cargo and passengers over long (avg. of 349.5 km) distances (lanchas). Road vehicles included public transit buses (combis) and group taxis (colectivos). Each vehicle was surveyed for *Ae. aegypti* adults (aspiration) and immatures (pupal surveys) at three different ports and two different bus/ taxi departure points. Our results show that *Ae. aegypti* adults and immatures are most prevalent on lanchas (13/17, 76.5%), lanchitas (5/15, 33.3%), and combis (3/16, 18.8%), while the remaining vehicle types were negative (Fisher's exact test across all vehicle types $p<0.0001$). Our data suggest that large, slow-moving river boats are much more likely than other types of vehicles to contain mosquitoes in general and *Ae. aegypti* mosquitoes specifically. Furthermore, it is evident that there is constant *Ae. aegypti* introductory pressure from Iquitos to riparian rural communities. Additional studies are underway to determine the role of local ecological conditions in the range expansion of the *Ae. aegypti*.

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REEMERGENCE OF *ANOPHELES FUNESTUS* AS A VECTOR OF *PLASMODIUM FALCIPARUM* IN WESTERN KENYA

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Malaria causes significant morbidity and mortality in western Kenya despite public health efforts to reduce its prevalence. Historically, the primary malaria vectors in the region have been *Anopheles funestus*, *An. gambiae s.s.* and *An. arabiensis*. Of these species, *An. funestus* populations declined the most after the introduction of insecticide treated bed nets (ITNs) in the 1990s in Asembo, and collections of *An. funestus* in the region remained low until at least 2005. We investigated indoor resting densities, human biting rates, and malaria infection rates of *An. funestus* and other malaria vectors in Asembo to determine if *An. funestus* populations and malaria transmission rates remained low or had increased despite intensification of ITN distribution. Additionally, we measured the sensitivity of the vector populations to pyrethroid insecticides in LLINs through standardized bioassays. We sampled *Anopheles* mosquitoes using the pyrethrum spray catch method (PSC) in 2010 and 2011 and the human landing catch method in 2011 and identified all specimens to species using both morphological and molecular methods. We tested female *Anopheles* for *Plasmodium falciparum* sporozoites by ELISA and identified blood meal hosts by direct sequencing of the vertebrate mitochondrial cytochrome B gene. We performed insecticide susceptibility assays on wild caught *Anopheles* adults collected from Asembo by exposure to permethrin, deltamethrin and bendiocarb. Contrary to findings during the early years of ITN use in Asembo, the majority of the *Anopheles* collected in our study were *An. funestus*. Female *An. funestus* had characteristically high *P. falciparum* sporozoite rates and showed nearly 100% anthropophily. Female *An. funestus* were found more often indoors during HLC sampling and had relatively low mortality rates during insecticide bioassays. Together, these results are of serious concern for public health in the region, indicating that *An. funestus* may once again be contributing significantly to the transmission of malaria in this region despite the relatively widespread use of ITNs.

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DETERMINING THE OPTIMAL MIX OF MALARIA CONTROL INTERVENTIONS IN THE HIGHLANDS OF WESTERN KENYA THROUGH SIMULATION MODELS

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Tools to assist malaria control professionals in their decision making processes will become increasingly important as calls continue for better targeting for malaria control interventions. A stochastic simulation modelling platform, OpenMalaria, was used to simulate the impact of combinations of a range of existing and potential future malaria control interventions implemented in the highlands of western Kenya. Combinations of interventions to include in simulations, as well as their coverage levels and deployment schedules, were chosen in collaboration with malaria control personnel in the study area to correspond to a 2011-2012 intervention evaluation trial. The model and baseline scenario were parameterized based on a previously-published, validated model of malaria epidemiology and control in the study area. Simulations ran for a period of five years on a population of 1,000 individuals using an ensemble of 14 model variants to address model uncertainty. The impact of intervention combinations was evaluated by estimating, after five years of implementation, the simulated annual average averted cases of uncomplicated malaria in children under five, reduction in all-age average parasite prevalence, and reduction in annual average EIR compared to the corresponding simulated outputs of the baseline scenario. The combination of interventions resulting in the largest simulated reduction in all indicators was long lasting insecticide treated net (LLIN) use by 80% of the population, 90% of households covered by indoor residual spraying (IRS) with deployment starting in April, and a mass screen and treat program covering 80% of schoolchildren implemented twice per school term. Despite moderate observed use in the population, simulations show LLINs and not IRS account for the majority of impact on transmission. While deployment of IRS starting in April ahead of the rainy season resulted in the largest simulated reduction in annual average EIR, starting IRS in May showed a greater impact on averting uncomplicated cases in children under five and reducing all-age parasite prevalence. These results have the potential to assist malaria control program managers in the study area if they wish to add new or change the implementation of current interventions.

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HOUSE ENTRANCE AND EXIT MOVEMENT PATTERNS, HOST PREFERENCE, AND WING GEOMETRY OF TWO FIELD POPULATIONS OF *ANOPHELES DARLINGI* ROOT THAT REPRESENT TWO GENOTYPES

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Anopheles darlingi Root, a major vector for malaria in Central and South America, has been shown to have two distinct genotypes: a northern lineage (Belize, Guatemala, Colombia, Venezuela and Panama) and a southern lineage (Amazonia and southern Brazil). To test whether behavioral differences in house entrance and exit movement patterns and host preference could be observed between each genotype, two field populations of *An. darlingi* that represented each genotype were observed. In Cayo District, Belize (representing the northern lineage), peak house entry occurred between 7:00-8:00 p.m. and 5:00-6:00 a.m. and peak exit occurred between 7:00-8:00 p.m. In Loreto Department, Peru (representing the southern lineage), peak house entry occurred between 10:00-11:00 p.m. and peak exit occurred between 11:00-12:00 a.m. Entrance and exit behavioral patterns were significantly different between the two populations of *An. darlingi* [log-rank (Mantel-Cox) $P < .001$]. The Belize population of *An. darlingi* was observed to have a significantly higher number of mosquitoes collected from a house with a human host than from a house with a pig host ($P < .025$). In Peru, there was no significant difference in the number of *An. darlingi* collected from a house with a human host and a house with a pig host. *Anopheles darlingi* collected from each experiment were analyzed using geometric morphometrics to compare wing shape and showed statistically significant shape variation between each geographic population ($P < .001$). A subset of *An. darlingi* samples from each site was sequenced to verify the genotype of each population. Information from these studies can be used to assess the relationship between genotype and host-seeking behavior and can be used for regional vector risk assessment.

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A NEW LONG LASTING INSECTICIDAL NET (LLIN) THAT COMBATS PYRETHROID RESISTANCE IN MOSQUITOES

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The large control programmes around the world and especially in Africa to combat malaria and reduce deaths predominately in children of >5

years old have employed Long Lasting Mosquito Nets (LLINs) which contain pyrethroid insecticides and are wash-proof for >20 washes. These have been largely responsible for reducing malaria deaths from c2 million/ year to <670,000. However pyrethroid resistance is increasing especially in parts of Africa and indications are that it is reducing the efficacy of this intervention. LLINs only use pyrethroids since low toxicity is essential due to the proximity of the people and especially children sleeping under them, therefore alternatives are limited. It has been known for many years that the synergist piperonyl butoxide (PBO) has a good impact on the metabolic based resistance mechanism in mosquitoes and is routinely used in household aerosols and space sprays, however it had not been used on nets due to the difficulties with stability and obtaining parallel degradation and loss curves with the pyrethroid over time. This new product Olyset Plus® has after much research and development achieved this and it contains both permethrin and PBO within the polyethylene fibres on all 5 surfaces of the rectangular net.The mesh size is smaller so it can be used against sandflies and the surface regeneration time of the actives has been speeded up the so there is minimal time after washing before it becomes active again. Trials have been conducted in both the laboratory and field against susceptible and multi-resistant mosquitoes. In comparison to a similar net without PBO the Olyset Plus performed much better even against susceptible mosquitoes due to faster penetration of permethrin through the cuticle aided by the PBO acting as a solvent and the suppression of P450 enzymes present naturally in susceptible mosquitoes. Independent trials conducted in both Benin and Cameroon showed excellent impact against both metabolic resistant population of mosquitoes and in Benin ones showing (Knockdown resistance) Kdr which was surprising.

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LLINS AND IRS ALONE WILL NOT ELIMINATE MALARIA IN THE SOLOMON ISLANDS: THE EVIDENCE TO SUPPORT NOVEL INTERVENTION USE

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The Solomon Islands are undertaking intensified malaria control and localised elimination. The key interventions are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), which work by killing mosquitoes after they enter houses. We therefore studied the biting behaviour of the primary malaria vector: An. farauti. This research was conducted in Central Province during 2011-2012. *Anopheles farauti* were observed to bite humans outside of houses (65% of the time) and early in the night (with 82% of biting before 9PM). After adjusting for when humans were inside houses, the percentage of exposure to mosquito bites which occurred indoors was only 16%. Mark-release-recapture experiments were then conducted to determine if the phenotypes for early and outdoor feeding are fixed in individual mosquitoes. Analyses showed that the phenotypes for time and place of feeding in consecutive feeding cycles were not significantly different from that observed for the entire population, consistent with a hypothesis that there is no structuring of the population. Hence, all individual An. farauti exhibit an equal probability to enter houses, and thus be exposed to the insecticides in LLINs and IRS during each feeding cycle. However, as *An. farauti* mainly bite outdoors and early in the night the efficacy of vector control tools deployed inside houses, such as LLINs and IRS, is significantly reduced. As such, there is an urgent need for complementary vector control tools that can target mosquitoes that feed outdoors and early in the night.

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SPATIO-TEMPORAL ANALYSIS OF LEPTOSPIROSIS INCIDENCE IN A HIGH-RISK URBAN SLUM COMMUNITY IN BRAZIL

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Leptospirosis has emerged as an important health problem in slum settlements worldwide. Yet, the lack of prospective information on transmission sources in these settings has hampered the development of effective interventions. A cohort of 2,003 residents from an urban slum community in Salvador, Brazil was recruited in 2004 and followed for a four-year period. Household interviews and surveys were performed annually to evaluate risk behaviors and geocodify place of residence and potential transmission sources. Annual serosurveys were performed to identify incident leptospiral infections. We fit a multivariate model that included random effects accounting for unexplained inter-person, spatial and temporal variation, whose structure can help to identify anomalous areas of high or low risk that can be further investigated. A total of 1094 individuals were followed for 4 years; 1724 completed at least one year of follow-up. The annual infection rate was 43.24 per 1,000 persons (95% CI 38.04-48.97), in a total of 5573 follow-up years. We identified household elevation - an inverse proxy for flood risk (OR 0.98 per meter; 95% CI 0.96-0.99), and reported sighting of rats near the household (1.57; 1.09-2.27), as significant independent risk factors in a model which included also age, male gender and illiteracy as significant covariates. Furthermore, reported contact with floodwater was associated with lower infection risk (0.49; 0.28--0.84), while contact with both floodwater and mud was independently associated with a higher risk of infection (2.27; 1.13-4.71). Although there was substantial temporal variation in infection risk (range 1.92-11.04 per 1000 persons), regions of highest risk did not vary year to year during the study period, and the analysis identified discrete locations with consistently higher or lower risk than was explained by the model. In slum communities, deficiencies in the peridomiciliary infrastructure, such as open sewers and poor drainage systems serve as persistent sources for transmission of leptospirosis. Furthermore, efficient transmission appears to require the specific interaction between flooding and exposed soil, the environmental reservoir for leptospires. Together these findings suggest that there are defined "hot spots" for transmission within slum communities and that targeting these sites by isolating soil from flood run off may be an effective prevention approach.

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THE USE OF MOBILE HEALTH (MHEALTH) TECHNOLOGY IN AN ORAL CHOLERA VACCINATION CAMPAIGN IN RURAL HAITI

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In mass vaccination campaigns, large volumes of data must be managed efficiently and accurately. In an oral cholera vaccination (OCV) campaign in rural Haiti during an ongoing epidemic, we used a mobile health (mHealth) system to manage data on 50,000 participants in 2 isolated communities. Data were collected using Samsung Galaxy 7.0 Plus tablets by 50 enumerators and 20 supervisors. Teams pre-registered and distributed

vaccine cards to eligible residents in 9,517 households during a 13-day census in February 2012. Using the tablets' barcode-scanning function, we tracked participants by unique numeric barcodes on their vaccine cards. First stored on devices, data were then uploaded nightly via Wi-fi to a web-hosted database. During the campaign from April to June 2012, residents presented their cards at vaccination posts and their barcodes were scanned. We pre-loaded vaccinee data from the census on tablets to automatically populate the electronic form, shortening each vaccination interaction. During 40 days of vaccination, 45,368 people received a first OCV dose. Of those, 90.8% were documented to receive two doses. Nightly analysis of community coverage each day informed the next day's vaccination strategy. Toward the end of each phase, we generated case-finding reports allowing us to specifically identify those who had not yet been vaccinated. The tablets' GPS capability allowed us to map vaccine posts, population size and vaccine coverage, providing deeper understanding of the reach of the campaign. The tablets withstood natural elements and battery life was sufficient for daily use (external batteries were used as backup). The hardware and software were user-friendly enough for use by high school-educated staff. Though mHealth solutions require up-front financial investment and training, they reduce the need for manual data entry and paper forms, which are costly and increase the risk of error. The use of mHealth allowed accurate, fast and high quality data collection and a targeted vaccination strategy in an OCV campaign in rural Haiti.

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PAPER TEST CARDS FOR FAST FIELD SCREENING OF SUBSTANDARD AND FALSIFIED PHARMACEUTICALS

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Substandard and falsified pharmaceuticals are a growing global health concern rooted in the information asymmetry between sellers and buyers. This presentation describes a novel approach to bridging this information gap by allowing rapid chemical analysis of pharmaceutical dosage forms without requiring laboratory facilities or instrumentation. We have devised an inexpensive and easy to use paper test card for rapid field screening of some common antibiotics and TB medications. The cards are printed with different reaction areas and loaded with chemical reagents; the cost of manufacture is under $US 0.50 per card. To use the test card, a pill is swiped over the reaction areas and the bottom edge is dipped into water. The capillary flow of water through the device activates different reagents and transports them to the drug sample. The test results appear as a color "bar code" that indicates the presence or absence of specific functional groups and compounds; the results can either be read by eye or by software that evaluates a cell-phone image of the test card. In a double-blinded laboratory validation, the test cards detected the active pharmaceutical ingredients (APIs) ampicillin, amoxycillin, acetaminophen, ethambutol, isoniazid, pyrazinamide, and rifampicin with high sensitivity and selectivity. Excipients such as starch, chalk, and talc were accurately detected, along with formulations that included substitute APIs or adulterants. This presentation will focus on a new version of the paper test card that can quantify APIs in solid dosage forms using colorimetric, titrimetric, and ratiometric approaches. Data will be presented on the limit of detection and limit of quantification of beta lactam antibiotics in formulations cut with various excipients, and discuss the scale of a regional post-market testing program that would be necessary for reliable detection of substandard or falsified drugs.

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LINKING HUMAN AND ANIMAL HEALTH: A POPULATION BASED ANIMAL SYNDROMIC SURVEILLANCE STUDY

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Livestock production is the main source of livelihood for over 70% of the rural population in Sub-Saharan Africa. In smallholder production system, livestock are kept in close proximity to humans sharing same environments, increasing probability of zoonotic pathogen transmission. However there is a dearth of data on the socio-economic impact diseases have on small-scale animal ownership, and the impact zoonotic diseases have on human health. To remedy this, a study conducting a simultaneous multi-year syndromic surveillance in humans and their animals in 1600 rural households in Western Kenya is being conducted. Each of the study household is visited at least bi-weekly and data on four human syndromes; fever, jaundice, diarrhoea and respiratory illness (cough, pneumonia), and 9 animal syndromes; respiratory, death, reproductive, musculoskeletal, nervous, digestive, skin disorders, udder disorders, and urogenital syndromes collected in cattle, sheep, goats and chicken are collected. Additionally, a comprehensive socio-economic survey is conducted in each of the 1600 households quarterly. Preliminary results show 80% of the study households own cattle, 88% own chicken, 62% own goats and 39% own sheep. Digestive syndromes, mainly diarrheas are the most common syndromes observed in cattle, goats and sheep, and account for about 50% of the livestock syndromes. From these data, we will determine if disease/syndromes in humans and animals cluster in certain households and consequently determine factors associated with high disease burdens. Animal syndromes with the greatest economic impact on the human health and human syndromes impacting on animal production will be identified. This study provides a unique dataset directly linking human and animal health, and socio-economic status at the household level. Such data will increase our understanding of the health implications of livestock keeping, and provide information vital to policy makers in setting priority and strategy on integrated human-animal disease control.

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EVALUATION OF CHROMATOGRAPHY PAPER AS A LOW-COST MEDIUM FOR ANEMIA DIAGNOSIS

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Anemia affects a quarter of the world's population, and a lack of appropriate diagnostic tools often prevents treatment in low-resource settings. Though the HemoCue 201+ is an appropriate device for diagnosing anemia in low-resource settings, the high cost of disposables ($0.99/test in Malawi) limits its availability. The low-cost WHO Hemoglobin Color Scale system ($0.02/test in Malawi) suffers from low accuracy. To address these concerns, we have developed a method that uses spectrophotometric measurement of blood spotted on chromatography paper as an accurate, low-cost (<$0.01/test) alternative to these methods. We optimized impregnating paper with chemicals to lyse red blood cells, paper type, drying time, wavelengths measured, and sensitivity to variations in volume of blood by using pipettes to apply blood to the paper and a laboratory spectrometer to take measurements. Lysing the blood

cells with sodium deoxycholate dried in Whatman Chr4 chromatography paper gave repeatable results, and the absorbance difference between 528 nm and 656 nm was stable over time in measurements taken up to 10 min. after sample preparation. The method was insensitive to the amount of blood spotted on the paper over the range of 5 µL to 25 µL. We created a low-cost, handheld reader to measure the transmission of paper cuvettes at these optimal wavelengths. Training and validating this device in a laboratory setting with patient samples on the handheld reader showed that the method is accurate to within 2 g/dL of the HemoCue device for 95% of samples. The measurement takes 6 seconds and can be performed at the patient's bedside. Field trials are planned for the summer of 2013 to evaluate our method in pediatric patients at the Queen Elizabeth Central Hospital in Blantyre, Malawi (n = 70) and in pregnant women at health clinics in Ventanilla and Cusco, Peru (n = 200).

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MOSQUITOES MEET MICROFLUIDICS - HIGH-THROUGHPUT MICROFLUIDIC TOOLS TO STUDY FIELD ECOLOGY OF INSECT-BORNE INFECTIOUS DISEASES

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Just as high-throughput tools and measurements at single-cell or single-molecule resolution have impacted our fundamental understanding in biology and medicine, physics-based precision measurement tools can also be applied to study field ecology of infectious diseases. We propose a novel high-throughput ultra-low-cost microfluidic tool to enable large-scale ecological measurements in wild insect vector and pathogen populations. The proposed microfluidic device is a 2D paper-based matrix of collection pockets, each sized to isolate an individual insect bite. The device is baited and placed in a field site to collect saliva samples when bitten by insect vectors. After collecting bite samples, reagent is supplied and the chip thermally cycled to detect bite locations and identify bites containing pathogens of interest, using multiplexed Taq-Man qRT-PCR. This technique is applicable to all diseases that are transmitted via saliva of an insect vector. Current work on the device uses the *Culex pipiens* vector and West Nile virus as a model system. We have collected discrete salivary droplets preserved in a 2D matrix by allowing *Cx. pipiens* mosquitoes to probe agarose gels. A 234bp fragment of the Ace2 gene specific to *Cx. pipiens* was amplified from these droplets through PCR. We have constructed a paper-based microfluidic device that can process 20000+ nanolitre-volume reactions on a 75mm by 25mm area (equivalent to a microscope slide), reducing reagent costs by an estimated 50,000 times. With this new technique, spatial and temporal population data at single-vector resolution can be collected anywhere in the world for monitoring malaria, dengue, West Nile Encephalitis, or any other mosquito borne diseases. This technique can be used to quantitatively measure distribution of pathogens in a vector population, for a better understanding of vector-pathogen interactions and population dynamics at scales never before possible. The device presents a low-cost, scalable solution to enable large-scale vector screening efforts worldwide. This can enable the scientific community to explore new frontiers in insect-borne infectious disease research, such as automated construction of high-resolution vector surveillance maps around the world, understanding the influence of climate and seasonal factors, or application of molecular techniques to understand evolution of pathogens or insecticide resistance in real world settings.

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A GEOSPATIAL ANALYSIS OF HEALTH CARE ACCESSIBILITY IN KENYAN HIGHLANDS

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In malaria-endemic, limited-resource regions, such as the western Kenyan highlands, seeking and having access to appropriate anti-malarial treatment is crucial. An important determinant of health-seeking behavior is accessibility to health care services. People living within 1 hour (of travel time) of a health care facility are considered by the WHO, to have health care access. We evaluated affordability and availability in determining health care access and health-seeking behavior. The inverse relationship between distance to facility and use of health care services has been well established, but has largely been determined by Euclidean distance. As utilization is affected by multiple factors, we designed a more comprehensive measure by integrating opinions and habitudes from 863 consumers, 15 health care facilities, and 135 retailers such as chemists or general shopkeepers. A spatial analysis of normative walking time from participants' households to their nearest available healthcare facility was used to evaluate health-seeking behavior based on actual travel time (vs Euclidean distance or perceived travel time). In spite of living less than 1 hour of travel time from a hospital or clinic (maximum calculated time: 46 minutes), 34% of participants did not exclusively choose to seek treatment from these facilities. Patients consistently overestimated the amount of time it would take to walk to the nearest health facility. This is a barrier to self-efficacy and presents an intangible hindrance to positive health-seeking behavior. Of those who sought treatment exclusively from commercial facilities, 25% did so despite needing to travel for longer periods of time in order to reach them. Surveys of area health care facilities indicated that 40% of them exhausted their stores of artemisinin-based anti-malarials at least 1 or more times per month. This lack of consistent access to appropriate medications may indicate why patients choose to seek treatment from commercial facilities instead. About 10% of the patients indicated that they take no action upon malaria symptom onset; however, patients' perception of affordability was not associated with the decision to seek treatment thus signaling a need for increased education and outreach in these rural communities.

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STUDY *IN VITRO* OF *TAENIA SOLIUM* POST ONCOSPHERAL STAGE

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Taenia solium is a parasite that causes neurocysticercosis (NCC) in humans. After the *T. solium* oncosphere enters the brain, it develops into a cysticerci. As this happens, the parasite produces a variety of molecules, which modulate the host immune response in order to avoid parasite destruction. The stage between oncosphere and cysticerci is the post oncospheral (PO) stage, which has not yet been characterized or studied. Study of the PO stage is important as proteins released during the stage could be used to improve diagnosis or provide new targets for vaccine development. For this reason, the objective of this work is to study the morphology and expression of total proteins during the PO stage. In vitro-hatched oncospheres of *T. solium*, prepared by the sodium hypochlorite method and activated using artificial intestinal fluid, were incubated on human intestinal monolayer cell. The parasites were collected at 1, 15, 30, and 60 days of incubation. The morphology was directly observed by microscopy. The proteins expressed in the oncosphere, PO, and cysticerci stage were compared by immunoblot. On day one of incubation, the activated oncosphere was, on average, 20 µm in diameter. The size of PO stage increased in relationship to the day of incubation, reaching up to 2500 µm in diameter at 60 days of incubation. Additionally, the number of cells and the thickness of oncospheral tegument also increased. When inoculated into the brain of a rat, the PO stage became cysticerci. By immunoblot, the PO stage has some proteins from both the oncosphere

and cysticerci stages. However, the expression of the oncosphere proteins decreases and the expression of the cysticerci proteins increases when the PO stage increases in size. It is possible that PO stage changes the expression of these proteins to modulate the host immune response and avoid its own destruction. When we tested a pool of sera from patients with NCC using proteins from the PO stage, two bands reacted strongly in immunoblot. These proteins could be used to diagnose patients with NCC in a noninvasive and relatively simple manner. This is the first time *T. solium* PO stage has been characterized *in vitro* and the discovered protein expression and parasite growth behavior could be used for diagnosis and further treatment development.

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EFFICACY OF A TSOL18/ISA206V VACCINE CANDIDATE AGAINST PORCINE CYSTICERCOSIS

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Taenia solium is a zoonotic parasitic disease that affects pigs and humans. The adult stage lives in the small intestine of humans; eggs are released to the environment through human feces. Pigs are infected after ingesting human feces containing eggs or proglottids and developed the larval stage (cysticercosis) mainly in muscle tissues. If a human is accidentally infect with eggs by eating contaminated food or water, he/she would develop neurocysticercosis, the major cause of acquired epilepsy in developing countries. It has been postulated that the disease could be prevented and controlled by pig vaccination; however, current vaccine candidates, such as Tsol18 recombinant, demand elevated costs of preparation. Therefore, finding an affordable and effective vaccine candidate will be critical for disease elimination efforts. The present study aimed to test the efficacy for a similar recombinant vaccine candidate using a different adjuvant (TSOL18/ISA206V) sponsored by GALVmed, a not-for-profit global alliance which aimed to improve human lives by applying livestock vaccines. A randomized controlled trial (n=49) was designed and TSOL18/ISA206V vaccine efficacy was compared to previously reported effective vaccine TSOL18/QuilA (Australian vaccine). Vaccine efficacy was measured by vaccination followed by oral challenge of *T. solium* proglottid and evaluated by carefully carcass examination (gold standard). The TSOL18/ISA206V vaccine candidate presented high vaccine efficacy (83.3%) compared with the control group and showed no difference when comparing its results with TSOL18/QuilA (Australian vaccine) or the same vaccine with QuilA as the adjuvant. The presence of TSOL18-specific antibodies was assessed by ELISA test showing that all of the vaccinated pigs were positive after the second vaccination. TSOL18/ISA206V vaccine is a promising candidate for a marketable vaccine against porcine Cysticercosis. Further studies should be carried out to evaluate other aspect of the immunogenicity of TSOL18/ISA206V vaccine and its effectiveness and efficiency.

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NEUROCYSTICERCOSIS TREATMENT USING RAT MODEL WITH *TAENIA SOLIUM*

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Neurocysticercosis (NCC) is caused by *Taenia solium* larvae infection of the central nervous system (CNS). NCC is the leading cause of acquired epilepsy and seizure conditions worldwide, especially in developing countries where pigs are raised and pork is consumed. In this study, we used a novel rat NCC model infected intracranial with activated *T. solium* oncospheres to evaluate and compare intraparenchymal and extraparenchymal infections, and to determine whether the model is beneficial for evaluating NCC treatment schemes. The First objective was to compare intraparenchymal, and extraparenchymal infections using different numbers of activated oncospheres (30, 60, 90, 180, 360, 720). The animals were sacrificed four months after infection. We found that the location of the infection was a factor to developing cysticercus. The proportion of animals that developed cysticercus in the brain was higher in the intraparenchymal group than in the extraparenchymal group. We also found that the number of cysticercus per brain increased with the number of oncospheres. The second objective was to assess the efficacy of Praziquantel and Albendazole to treat NCC. Rats infected with *T. solium* oncospheres were treated four months after infection with Praziquantel (75mg/Kg/day) plus albendazole (15mg/Kg/day) during 3 days, followed by 7 days of treatment with albendazole (15mg/Kg/day) only. The animals were sacrificed one month after treatment. Using histochemical by H&E, we observed that the cysticercus from treated animals demonstrated a partially degenerated cyst, with not whole destroyed cyst wall with high inflammatory response surrounded by a thick layer of collagen type I. The control group (infected, untreated rats) showed cyst viable parasite (scolex) with intact vesicular cyst wall, minimal to moderate host reaction (inflammatory response) with only a thin layer of collagen type I, which was either intact or mingled with inflammatory infiltrate. These results imply that the rat NCC model could be a beneficial model for understanding the progression of NCC in humans, and provide useful information for treatment.

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THE MONETARY BURDEN OF CYSTICERCOSIS IN MEXICO

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Taenia solium cysticercosis is a major public health and agricultural problem in many developing countries where health education, sanitation, and meat inspection infrastructure are insufficient. Cysticercosis affects both human and animal health and has important economic consequences. Very few studies have been conducted to evaluate the monetary burden of cysticercosis. This study provides the first estimate of the monetary burden of cysticercosis in Mexico. The total monetary burden of cysticercosis, in Mexico, was estimated by assessing costs associated with infection of both humans and pigs. The cost of neurocysticercosis (NCC), in humans, took into consideration losses due to NCC-associated epilepsy and NCC-associated severe chronic headaches. Epidemiologic and

economic data were obtained from the published literature, government reports, and interviews and chart reviews of NCC patients treated at two neurological referral hospitals located in Mexico City, Mexico. Latin hypercube sampling methods were employed to sample the distributions of uncertain parameters and to estimate 95% credible regions (95% CRs). The overall monetary burden of human NCC, for Mexico, was estimated at U.S.$71,267,032(95% CR U.S.$44,470,935 - U.S.$103,235,886) per year, of which 45% was attributed to individuals with NCC-associated epilepsy, 39% was attributed to individuals with NCC-associated severe chronic headaches, and the remainder was attributed to NCC patients with both clinical manifestations. An additional U.S.$23,078,764 (95% CR U.S.$8,020,568 - U.S.$39,757,504) was estimated to be lost due to cysticercosis in the Mexican pig population annually. This study suggests that *T. solium* cysticercosis continues to result in considerable monetary losses to Mexico.

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MANAGEMENT OF CYSTIC ECHINOCOCCOSIS: THIRTY YEAR EXPERIENCE IN A SINGLE REFERRAL CENTER IN NORTHERN ITALY

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Cystic echinococcosis (CE) is a chronic, complex and neglected disease. In humans, its clinical spectrum ranges from asymptomatic infection to severe, occasionally even fatal disease. Four management options exist: surgery, percutaneous techniques and drug treatment for active cysts, and watch and wait for uncomplicated inactive cysts. However the four options have never been properly evaluated and compared, and the evidence base for clinical decision making is still limited. In this context, we describe our experience with clinical management of CE in a single referral center in North Italy over a time span of 30 years and look at lessons learned. Patients referred for confirmed or suspected CE from 1982-2012 were included. Data were available for 1022 patients and 1295 cysts. CE was confirmed in 695 patients (881 cysts), while the remaining 327 were non-parasitic cystic lesions. Patients with CE related symptoms were 418 while 277 (40%) were asymptomatic. Among 440 patients diagnosed with CE and monitored over time, 297 (67%) have been treated, while 143 (33%) have been managed expectantly. Of the 695 CE patients, 440 (63%) have been followed-up for a mean period of five years. Foreign patients (203, 29% of the total number) were mostly immigrants from the highly endemic areas of Northern Africa and Eastern Europe, and their number has steadily increased over the years. In our cohort, almost a third of the patients were managed expectantly, thus saving resources and avoiding unnecessary treatments. Ruling out non parasitic cystic lesion is an important part of diagnostic workup. The number of immigrants with CE has steadily increased in the last 10 years. CE patients should be managed in referral centers until guidelines on treatment with a strong evidence base become available.

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CENTRE-BASED CLINICAL MANAGEMENT OF CYSTIC ECHINOCOCCOSIS

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Neglected infectious disease (NIDs) play an increasing role in non-endemic countries at the current level of global mobility. Cystic echinococcosis (CE) is one of the world's most neglected NIDs. CE lesions, predominantly in the liver and lungs, develop silently over long periods of time until complications suddenly precipitate. In high-income countries mostly immigrants from CE-endemic areas are affected and health services are, as a rule, not experienced to diagnose, stage and manage CE patients appropriately. The setting and the impact of the interdisciplinary CE Centre at Heidelberg University Hospital is presented where infectious disease / tropical medicine physicians, radiologists, abdominal and thoracic surgeons, gastroenterologists and parasitologists work very closely together to stage CE patients (ultrasound-based cyst classification) and to tailor currently available treatment options (medical treatment with albendazole, percutaneous cyst-sterilization techniques, surgery and 'watch and wait') to the needs of the individual patient.

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IMMUNOBLOTTING WITH HUMAN ANTIGEN CORRELATES WITH CYST STAGE IN PATIENTS WITH CYSTIC ECHINOCOCCOSIS OF THE LIVER

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Cystic Echinococcosis (CE) is a zoonosis caused by infection with the larval stage of *Echinococcus granulosus*. Although the natural history of cyst development is not completely known, they likely pass through several stages, from active to inactive forms. The diagnosis of hepatic CE is based on ultrasonography, confirmed by a combination of immunodiagnostic tests. These, however, lack standardization and have low sensitivity and specificity. In addition, no serological marker is currently available which correlates with cyst viability or the ability to predict the evolution of a cyst, implying the need for years-long patient follow-up after treatment. We present a preliminary characterization of the performances of an immunoblotting (IB) test based on human hydatid cyst fluid (HCF) with particular regard to its ability to distinguish between cyst stages. Patients with active cysts (CE1 and CE2) responded differently to HCF: while patients with CE2 cysts consistently recognized subunits of CE major antigen AgB, these were inconsistently recognized by sera from CE1 patients. Patients with inactive cysts (CE4 and CE5) did not recognize any specific band. Finally, patients with transitional cysts (CE3a and CE3b) recognized subunits of AgB and Ag5. Most importantly, the experimental IB allowed in many cases to discriminate between CE3a and CE3b, known to have different viability profiles. In an attempt to assess whether the experimental IB could detect early changes in cyst viability, we tested it on sera from one patient at time-points where the hepatic CE cyst passed from active (CE1) to transitional (CE3a) to inactive (CE4) stages after albendazole treatment. We observed a rapid change in band pattern recognition. These findings strongly support the hypothesis that different antigens are expressed by different cyst stages, whose recognition might be useful in clinical practice to correctly define cyst viability and open new opportunities to develop diagnostic tools that could guide clinical decision-making and shorten patient follow-up.

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REACTIVE CASE DETECTION FOR MALARIA ELIMINATION IN SWAZILAND: FACTORS ASSOCIATED WITH THE DETECTION OF SECONDARY *PLASMODIUM FALCIPARUM* INFECTION

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Reactive case detection (RACD), the screening of household members and neighbors of passively detected malaria cases for infection, is recommended for malaria elimination but there is little evidence to guide

practice. We performed a prospective surveillance study in Swaziland to identify factors related to the index case or individuals screened associated with the detection of secondary cases. We also compared RDT to a molecular method, loop-mediated isothermal amplification (LAMP), for the detection of secondary cases. RDT and/or microscopy confirmed index cases reported from 294 health facilities in Swaziland were targeted for follow-up. If there was potential local acquisition (area receptive to malaria transmission or no travel history), family and neighbors residing within a 1 km radius were targeted for RACD. We collected dried blood spots (DBS), GPS coordinates, and information on demographics, travel, vector control, housing, season, area receptivity, response time and coverage of RACD, and index case clinical characteristics. Family and neighbors testing positive by RDT were referred for treatment; DBS from all subjects were tested by LAMP. Bivariate analyses of potential relationships between risk factors and secondary case detection by LAMP were performed using t-test or logistic regression. From 8/2012 to 4/2013, 165 index cases were identified; 56 qualified for and underwent RACD resulting in 1481 household members and neighbors screened. Secondary cases were more likely to occur when the index case was LAMP positive at follow-up (OR 6.9, 95% CI 1.3-36.8, median follow-up at 5 days, 95% CI 1-29), RACD was timely (within 4.9 days, 95% CI 2.7-8.8, vs. 11.6, 95% CI 8.3-16.1, p=0.009), and more subjects screened (mean 28 people, 95% CI 12-63, vs. 10, 95% CI 7-15, p=0.008). Among individuals screened, LAMP positivity was associated with travel outside Swaziland (OR 12, 95% CI 5.1-28.2) and closer distance to the index case (mean 39.8m, 95% CI 13.6-116.4, vs. 151.0m, 95% CI 134.6-169.4, p=0.001). To date, LAMP has detected 5 fold more infections than RDT (2.5%, 27/1093, LAMP positivity vs. 0.5%, 6/1093, RDT true positivity using LAMP as gold standard). Post-treatment LAMP positivity in index cases is likely due to gametocytes and points to a potential role for additional gametocidal agents to prevent onward transmission. The effectiveness of RACD to detect secondary infections can be improved using LAMP, optimizing response time, screening radius size, and target population.

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HIGH PREVALENCE OF ASYMPTOMATIC MALARIA IN URBAN SETTINGS IN DOUALA, CAMEROON

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Malaria remains a major health problem in Cameroon with 38% of consultations and 24% of deaths. The negative economical impact of malaria has encouraged a new approach targeting companies with counseling and distribution of prevention kits for workers and their families. A cross sectional study was undertaken from October 2012 to February 2013 to collect preliminary data to assess the impact of the Exxon Mobil foundation control program in enterprises and communities in Douala, which consisted of indoor spraying and distribution of Long Lasting Impregnated Nets (LLINs). 2191 people in six communities and 829 in three enterprises were interviewed and screened with a mass diagnosis method based on malaria rapid blood test using pre-stained slides for fluorescence microscopy (CyScope®, Partec GmbH, Germany). Alongside, 783 children were also screened in five schools. All positive cases were treated immediately. A high prevalence of asymptomatic malaria was determined with a mean of 38,02% in the screened population of 3803 individuals. 45,47%, 40,48% and 24,49% of malaria tests were positive in schools, communities and companies respectively. Only 1% of the positive cases had fever. The prevalence in schools correlated with sanitation indices. The highest prevalence in communities was registered among children under five (42,22 %). Out of 2494 persons who responded to questionnaires, 1614 owned a LLIN. This group was less affected by malaria infection than those without nets, although the difference was not significant. (36,93% against 38,52%; X2 = 0,61, ddl = 1, P>0,05). The average coverage was 3,10 persons/net. The impact of malaria control initiatives can be better assessed with the use of mass diagnostic tools. Asymptomatic malaria is highly prevalent in Douala but coverage with LLINs is still insufficient. This situation makes malaria elimination difficult to envisage in endemic areas. However, malaria elimination can be foreseen if all detected cases are promptly treated and concomitantly protected from anopheles bites during the lifespan of gametocytes in persons with parasitaemia.

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A 2012 DEMOGRAPHIC AND SEASONAL PROFILE OF HUMANS HOSTING THE MALARIA PARASITE RESERVOIR IN ZAMBIA: RESULTS FROM MASS SCREEN AND TREAT (MSAT) ACTIVITIES IN SOUTHERN PROVINCE AND IMPLICATIONS FOR ELIMINATION

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Control programs aiming for malaria elimination are tasked with clearing parasites from people and preventing transmission from mosquitos to humans. Documenting the human parasite reservoir and profiling demographic characteristics and seasonal variations can guide efforts to clear parasites from people and prevent transmission. Three rounds of MSAT in southern Zambia in 2012 provide this unique information. MSAT covering a population of approximately 90,000 residing in 4 districts was carried out during low transmission season (April to October). A rapid diagnostic test was administered to all individuals and infections were treated with artemether lumefantrine. District parasite prevalence during round 1 ranged from 3% to 44%; in round 2, from 5% to 22%; and in round 3, from 2% to 16%. Trends in parasite prevalence were highest across rounds among children age 5-14 years, and lowest among adults age 50+. Nearly half of the parasite reservoir consistently resides in children age 5-14. In comparison with population age structure, the reservoir disproportionately resides within this age group. Infants and individuals age 25 and above are disproportionately less likely to carry malaria infection. Results indicate need to target school age children, a finding in line with 2012 Malaria Indicator Survey (MIS) results indicating need to improve bed net usage among this age group. MIS results indicate that bed net usage is high until age 5 (57%), falls among school age children (age 5-9, 45%; age 10-19, 37%), and increases with age from age 20 to 49 (e.g. age 45-49, 61%). Another important characteristic of the parasite reservoir in this context is that it is largely characterized by afebrile infections. Infected individuals reporting recent fever ranged from 13% to 23% in round 1; 4% to 7% in round 2; and 2% to 8% in round 3. Afebrile infection is the norm across the lifespan, although older age (50+) is associated with higher likelihood of fever accompanying infection. These results highlight the importance of clearing parasites from asymptomatic (afebrile) people.

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EFFECTS OF MALARIA CONTROL ON THE HEALTH FACILITY: CHANGES IN COSTS AND HOSPITALIZATIONS IN TWO HOSPITALS IN ZAMBIA

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The intensive scale-up of malaria control efforts in recent years has significantly reduced the malaria burden worldwide. There is little evidence, however, of the impact of malaria control on broader health systems, particularly at the health facility level. We present a pre-post comparison of hospital admissions and outpatient visits for malaria before and after the scale-up of malaria control by using retrospective, longitudinal facility-level data and patient record data covering the period 2003 to 2008 from two hospitals in Zambia's Southern Province. We also conducted costing analyses to estimate the costs of testing and treating malaria patients by year at both hospitals to determine changes in the total financial resources devoted to malaria admissions over time as malaria control is scaled up and inpatient malaria admissions decrease. Results show a substantial reduction in inpatient admissions and outpatient visits for malaria at both hospitals following the scale-up. The proportion of total hospital visits for malaria decreases over time in both facilities. At one hospital, malaria admissions account for 20% of total admissions for patients under-5 before malaria control scale up, compared to 1% after malaria is controlled. Hospital spending on malaria admissions also decreased with the expansion of malaria control. In one hospital, malaria accounted for 11% of total hospital spending before malaria control scale-up compared to less than 1% of hospital spending following malaria control. The study findings demonstrate that as malaria is better controlled in the catchment area facility-level resources used for malaria treatment also decline, potentially freeing resources for the treatment of other conditions.

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RESPONSE TO ANTIMALARIAL THERAPY WITH ARTEMETHER-LUMEFANTRINE AMONG INFANTS RANDOMIZED TO THREE DIFFERENT ANTIMALARIAL CHEMOPREVENTION REGIMENS IN AN AREA OF HIGH TRANSMISSION INTENSITY

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The burden of malaria remains high for children in parts of Africa despite the use of insecticide treated bed nets (ITNs). Chemoprevention has the potential of reducing the malaria burden; however, limited data exist on the efficacy and safety of anti-malarial therapy in the setting of chemoprevention. A cohort of 400 infants aged 4-5 months were enrolled using convenience sampling in Tororo, Uganda, an area with perennial high transmission intensity, given an ITN, and followed for all their health care needs 7 d/wk. At 6 months of age, 393 infants were randomized using an open label design to one of four treatment arms; no therapy, monthly sulfadoxine-pyrimethamine (SP), daily trimethoprim-sulfamethoxazole (TS), or monthly dihydroartemisinin-piperaquine (DP). Study drugs were administered unsupervised at home until 24 months of age. Episodes of uncomplicated malaria were treated with artemether-lumefantrine (AL) and followed for 28 days. The risk of day 3 *parasitemia*, recurrent *parasitemia*, and adverse events was compared across chemoprevention arms using generalized estimating equations.767, 734, 618, and 368 episodes of malaria were treated in the no therapy, SP, TS, and DP arms, respectively. Only 21 of 2487 (0.8%) treatments were for complicated malaria (12 danger signs and 9 severe malaria). Following treatment for uncomplicated malaria with AL, 99.3% achieved parasite clearance by day 3 and the cumulative risk of recurrent *parasitemia* after 28 days was 46.0% and did not differ across the 4 chemoprevention arms (range 44.7-47.0%). Compared to the no therapy arm; the SP arm was associated with a higher risk of jaundice (1.5% vs. 0.1%) and a lower risk of diarrhea and vomiting; the TS arm was associated with a lower risk of cough, diarrhea, anorexia, and anemia; and the DP arm was associated with a higher risk of neutropenia (16.6% vs. 10.0%) and a lower risk of anemia. The risk of complicated malaria was very low in this cohort of infants living in a high transmission setting. Treatment of uncomplicated malaria with AL was associated with excellent parasite clearance and treatment efficacy and safety were not significantly influenced across the range of chemoprevention regimens.

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IMMUNE PROTECTION AFTER MALARIA CEASED ON ISLANDS

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Re-infection is a major concern after malaria elimination. After weekly mass drug administration for nine weeks combined with vector control on the entire population (718) of Aneityum island in 1991, *Plasmodium falciparum* disappeared and *P. vivax* from 1996 onwards. Transmission interruption was sustained until a *P. vivax* epidemic was reported in January 2002. We aimed to assess age-specific malaria prevalence during this epidemic. Two cross-sectional malariometric surveys of the entire population of Aneityum were conducted six and ten months after the index cases. In July 2002 *P. vivax* infections were detected by microscopy in 22/759 individuals: 20/298 born after beginning the elimination programme in 1991, 2/126 between 1991 and 1982, and 0/339 before 1982. PCR diagnosis increased the infection number to 77 distributed amongst all age groups. Parasite prevalence was 12·1%, 16·7%, and 6·0%, respectively. In November a similar age pattern was found but with fewer infections: 6/746 and 39/741 by microscopy and PCR, respectively. All microscopy positive cases were PCR positive. Antibody responses to *P. vivax* were significantly less for individuals born after 1991 than for older age groups. A remarkably low genotype diversity of immune target antigen genes *Pvmsp1* and *Pvcsp* observed in Aneityum (h= 0·15) when

compared with the other islands (h= 0·89–1·0) suggests a recent parasite re-introduction was linked with malaria resurgence. Acquired immunity against *P. vivax* persists after malaria exposure has ceased and appears to protect individuals born before elimination from clinical disease. The immunity does not prevent infection per se but suppresses erythrocytic stage infection to sub-microscopic levels. The stable and limited antigen SNPs on islands may likely underline anti-malarial immune protection against reinfections in Aneityum adults.Protection is required to prevent young populations from clinical diseases, but interventions including all populations remain critical to sustain malaria elimination.

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GENETIC SURVEILLANCE DETECTS BOTH CLONAL AND EPIDEMIC TRANSMISSION OF MALARIA FOLLOWING ENHANCED INTERVENTION IN THIÈS, SENEGAL

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Using whole genome sequencing information we developed a molecular barcode tool that queries independent single nucleotide polymorphisms from the *Plasmodium falciparum* genome Genotyping data obtained from screening patients with mild uncomplicated malaria seeking treatment at a clinic in Thiès, Senegal from 2006 to 2011 revealed an increasing frequency of infections caused by genetically identical parasite strains representing 10% of the population in 2006 and more than 50% of the sampled population in 2011 that coincided with growing deployment of malaria control interventions and decreased malaria deaths. Several of these parasite genotypes persisted clonally across different transmission seasons. We also observed an increase in the frequency of genetically identical parasite strains corresponded with a decreased probability of multiple infections. We performed a pilot survey of asymptomatic individuals from the same clinic catchment site and observed a similar frequency in the number of shared barcodes among this population indicating that specific parasite types were not specifically associated with illness in the passive case detection population. We are addressing whether immunity plays a role by looking at the distribution across the age spectrum and are investigating whether patients harboring parasites with shared barcodes have a higher gametocyte carriage rate that may imply they are more easily transmitted in the population. Analysis of these trends support evidence of both clonal and epidemic population structures. These data provide the first evidence of clonal parasite populations in Africa emerging after deployment of substantive malaria-reducing efforts including bednet distribution, use of rapid diagnostic tests for case detection, and treatment with artemisinin combination therapy. We hypothesize that reduced malaria transmission results in decreased outcrossing in the mosquito midgut that results in emergence of clonal parasite types when transmission becomes sufficiently reduced. This implies that genetic surveillance with genotyping tools that assess these changes in parasite population structure can be used to evaluate the effectiveness of disease control strategies and assist a rational global malaria eradication campaign.

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CASE INVESTIGATION AND REACTIVE INFECTION DETECTION FOR MALARIA ELIMINATION IN NORTHERN SENEGAL

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A malaria case investigation program was piloted for 12 weeks in 2012 in Richard Toll district of northern Senegal. Malaria infections (N=110) were identified through facility-based passive case detection and investigated within 3 days. RDTs and a brief questionnaire were administered to 5520 individuals (average of 50 contacts per index case) living within the index case compound or within 5 neighboring compounds. In comparison with family and neighbors, index cases were more likely to be male, age 15-49, employed outside of the home, and to report recent travel. Twenty-three (0.4%) of the family/neighbors were RDT positive. Potential risk factors for infection among family and neighbors were examined including: sex, age, occupation, travel history, bed net usage, and residence (index vs. neighboring compound). Adjusting for all factors, risk of infection was associated with recent travel and residence in the index case household. RDT positivity was notably high among people with recent travel to Dakar (10.5%) or other regions in Senegal (33.3%) as compared with those who did not report such travel (0.2%). Recent fever among RDT-positive family and neighbor contacts was uncommon (30%). We examined possible screening criteria that would optimize the efficiency of contact investigations in this population. Rather than testing all 5520 people in index and neighboring compounds to identify 23 infections, if testing was targeted to all people in the index compound and only neighbors who report recent travel or fever, only 1173 individuals would be tested to identify 20 of the infections. The remaining 3 cases not identified by screening for recent fever or travel would have been identified by including screening questions regarding boarding at school outside of the region. Expanding and optimizing case investigation with specific targeted testing and treatment of at-risk contacts can facilitate continued progress towards malaria elimination in northern Senegal.

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SPATIO-TEMPORAL PATTERNS ASSOCIATED WITH DELIVERY OF A MASS SCREEN AND TREATMENT CAMPAIGN IN SOUTHERN ZAMBIA: IMPLICATIONS FOR MODELING SYSTEM PARAMETERS

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The Zambia National Malaria Control Program has successfully scaled-up recommended malaria control interventions over the past decade and is pursuing alternative methods to further reduce malaria transmission including community-targeted, parasite reservoir reduction strategies. During the 2012 dry-season (June-November) in Southern Zambia, three large-scale mass-screen-and-treat (MSAT) rounds were undertaken with rapid diagnostics tests (RDT) and the anti-malarial drug, artemether-lumefantrine. Randomized health facility catchment populations were visited at their homes in a full community census and, following individual consent, were administered rapid diagnostic tests and RDT-test

positive individuals were provided treatment according to national policy recommendations. Using the surveillance data collected during these activities, we analyze spatio-temporal patterns and extract parameters relevant to modeling the system and its response to intervention campaigns. These include data-driven estimates of programmatic coverage rates, the extent of internal migration within the study area, the underlying age- and exposure-specific immunity, and the potential effects of heterogeneous biting and non-compliant usage of distributed drugs and ITNs on re-infection risk. As malaria control and elimination efforts progress, optimizing the combination of prevention and treatment strategies for delivery at community level are essential to reduce the transmission potential among those asymptomatic carriers.

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HEALTH FACILITY INCIDENCE OF SEVERE MALARIA AND PNEUMONIA IN CHILDREN UNDER FIVE, FOLLOWING A RANDOMIZED INTEGRATED COMMUNITY TRIAL IN EASTERN UGANDA

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In Africa, malaria and pneumonia are among the leading killers of children under five. The overlap of clinical presentation between the two diseases poses a challenge especially for clinicians with limited diagnostic tools. The Integrated Community Case Management (ICCM) of Childhood Malaria, Pneumonia and Diarrhea has been adopted by many African countries including Uganda. A randomized controlled community trial was conducted in Eastern Uganda, in which integrated care for malaria and pneumonia was provided in intervention and control villages. Under five children in intervention villages received Coartem (CoA) for the treatment of malaria and amoxicillin (Amox) for pneumonia while children in control villages only received CoA for malaria. This post-intervention study assessed the incidence of severe malaria and pneumonia at health facilities in the study area, before and after intervention. Health facility incidence of severe malaria and pneumonia was used as a proxy measure of severe incidences for the two diseases at community level. Health facility records over a 5 year review period (n=10,236) on severe malaria and pneumonia in children under five were abstracted using a health facility tool based on WHO clinical classification for severe malaria and pneumonia. Their incidences were compared before and after study intervention, and between intervention and control study villages. The incidence of severe malaria in study villages was 943/20770 (4.5%) before intervention, compared to 999/22674 (4.4%) post study intervention (OR=0.97, 95% CI [0.89 -1.06] p value 0.52). Incidence of severe pneumonia was 32/2223 (1.4%) before intervention compared to 53/2714 (2.0%) after intervention (OR=1.36, 95% CI [0.85 -2.16], p value 0.17). After intervention, the incidence of severe malaria from intervention villages was 4.4% compared with 17% from non-study villages (OR=0.26, 95% CI (0.24-0.28) P value <0.001). Following intervention, the incidence of severe pneumonia was 2% intervention villages compared with 22.7% for non-study villages (OR=0.47, 95% CI (0.39-0.57) P value<0.001). Overall, the proportion of children presenting with severe morbidity due to malaria and pneumonia was much lower in study villages after intervention, compared to non-study villages.

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EVALUATION OF THE TOLERANCE OF SULFADOXINE-PYRIMÉTHAMINE + AMODIAQUINE COMBINATION IN SEASONAL MALARIA CHEMOPREVENTION (SMC) COMBINED WITH HOME BASED MANAGEMENT (HMM) IN CHILDREN UNDER 10 YEARS IN SENEGAL

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Seasonal Malaria Chemoprevention (SMC) is an important tool for malaria prevention in children in sub-Saharan areas where transmission is intense and seasonal. It showed superior efficacy to 70% with AQ + SP combinations. Nevertheless, the data related to the tolerance of the products remain low and access to health facilities is an obstacle to the notification, the objective of this study was to assess the safety of SP¨+AQ when administered by Community Health Workers (CHWs) and home care Providers living in the same villages as mothers who report adverse events (AEs). The study was conducted in the health district of Saraya located in the south-eastern of Senegal. The CHWs/Providers, community supervisors and qualified district staff had been trained in recognition of AEs related to SP and AQ. During the last two cycles of the intervention a child sample was selected and their mothers visited the 4th day to assess the proportion of children who vomited the second and third doses administered at home. Every two weeks supervisions were conducted to collect tools. In total, 40 officers were trained and 33000 doses administered between July and November 2011, 29 notifications were recorded, including 14 due to SMC. Vomiting was more reported with an incidence of 37.9% and 1 case of rash was recorded. Most of rejection of drugs was notified causing a decline in coverage in a village. This decrease was corrected through social mobilization. A decline in notifications was noted over time. No case of serious AEs was recorded. The involvement of CHWs/Providers is important in the notification and the possible detection of severe cases for their proximity to children receiving SMC under implementation.

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CLUSTER RANDOMIZED TRIAL OF AN INNOVATIVE PAY-FOR-PERFORMANCE (P4P) STRATEGY TO IMPROVE DIAGNOSIS AND TREATMENT OF MALARIA IN WESTERN KENYA

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In rural health facilities without malaria diagnostic tests, as many as 90% of febrile patients receive an antimalarial. Moreover, in facilities with testing services, 40-80% of patients with a negative test receive an antimalarial. This practice leaves the true cause of fever untreated, accelerates the spread of drug resistance, and wastes costly drugs. Pay-For-Performance (P4P) programs have generated interest as a potential mechanism to improve health service delivery and accountability. However, there has been little experimental evidence to assess the effectiveness of P4P programs in developing countries. We describe a cluster-randomized controlled trial underway in 18 health centers in western Kenya testing an innovative incentive strategy to improve diagnosis and treatment of malaria. The incentive scheme promotes adherence to WHO guidelines for laboratory confirmation of malaria before treatment. There are three important innovations to this study: the behavior being incentivized is quality of care rather than volume of service; the incentives are applied at the facility rather than individual level, thus benefiting overall facility infrastructure and performance; and the incentives are designed to be budget-neutral. Following clinical and laboratory training and

establishment of a monthly EQA for malaria microscopy, the percent of malaria cases confirmed by laboratory diagnosis increased from 25% to 52% while slide positivity decreased from 30% to 20%. The mean sensitivity and specificity for a six-month period across the 18 facilities was 98% and 85%, respectively. Monthly artemether-lumefantrine consumption decreased by 34% between the period prior to the training and the period following the training. We will also discuss the effect of the intervention on malaria testing and prescription practices, as well as projected cost savings, after one year of incentives. This study will demonstrate whether facility rather than individual incentives are compelling enough to improve case management, and whether these incentives lead to cost-savings due to reduced drug consumption.

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USE OF INSECTICIDE QUANTIFICATION KITS (IQK) TO INVESTIGATE THE QUALITY OF SPRAYING AND DECAY RATE OF BENDIOCARB ON DIFFERENT WALL SURFACES IN KAGERA REGION, TANZANIA

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Bendiocarb was introduced for Indoor Residual Spraying (IRS) in Tanzania in 2012 as part of insecticide resistance mitigation. This study aimed to monitor insecticide concentration to assess 1) intra-operational IRS coverage and quality of spraying and 2) decay rate of insecticide on different wall surfaces. The study was conducted in Muleba and Karagwe districts. Wall substrate samples were obtained by scratching wall surfaces using a scalpel and collecting it in eppendorf tubes. To assess intra-operational IRS coverage and quality of spraying, 102 houses were randomly selected. A total of 510 samples (218 in Muleba and 292 in Karagwe) were obtained for IRS coverage and intra-spraying quality assessments. To investigate decay rate, 30 houses in Karagwe with recommended concentration at baseline were selected. The wall substrates included: burnt bricks (five); cement plastered (three); mud wall (twenty); and lime plastered walls (two). Follow-up samples were collected on monthly basis for over a period of five months. Laboratory testing of insecticide was done using the Carbamate Insecticide Quantification Kit (IQKTM [Innovative Vector Control Consortium, www.ivcc.com]). The IQK assay is based on inhibition of the activity of recombinant acetylcholinestarase (AChE) by bendiocarb which is dependent on the concentration of insecticide. Of the 510 samples, 89.4%(95% confidence interval [CI]86.4-91.9) met the WHO recommended concentration for IRS coverage. The proportion of houses meeting WHO standards for IRS coverage varied between Muleba (96.3%) and Karagwe (84.2%) (p-value<0.001). Bendiocarb decay follow-up in Karagwe showed that the proportion of houses with recommended concentration (between 100 - 400 mg/m2) declined from 93.3%, 92.6% and 73.1% at months one, two, and three post IRS, respectively (ptrend=0.03). The acceleration of decay increased in fourth and fifth month post IRS with WHO standards met in only 30.1% and 7.7% of houses, respectively. All house surfaces meeting WHO standards at month five were made of burnt brick walls. IQK is an important tool for assessing IRS coverage and quality of spraying, and can monitor insecticide decay over time to establish the right time to conduct a new spray cycle.

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IMPROVING GLOBAL FUND PROGRAMMATIC INDICATOR PERFORMANCE - KENYA, 2012

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Kenya received a Global Fund (GF) Round 10 grant to strengthen national malaria control activities. GF disbursed $7.8 million, including $5.4 million for commodities, to Kenya in 2012. In October 2012, the overall grant performance was rated "unacceptable" (i.e., C rating). We subsequently implemented two strategies to address programmatic performance. We integrated malaria commodities data reporting, including rapid diagnostic test and artemether-lumefantrine treatment, into the routine District Health Information System (DHIS2) to ensure all malaria indicator data was reported monthly in a single system. The President's Malaria Initiative also supported a modest one-time reimbursement (up to $175) for facilitated supervision expenses incurred by district pharmacists to collect malaria commodities data from non-reporting facilities via telephone, short message service or visits and enter the data into DHIS2. Reporting of malaria commodities data by health facilities via the legacy Logistic Management Information System averaged 40% (range 37-45%) per month between January-September 2012. Reporting via DHIS2 increased to 72% (range 71-72%) per month between October-December 2012. The GF core programmatic indicator, the percent of target reached for number of people treated appropriately for malaria, averaged 40% (range 25-56%) per quarter between January-September 2012. The GF core programmatic indicator increased to 92% for the last quarter from October-December 2012. Ninety of 285 (32%) district pharmacists requested reimbursement; reimbursements totaled $15,600 or 0.3% of commodity costs. Implementation of two strategies, integration of malaria commodities data into DHIS2 and facilitated supervision by district pharmacists, dramatically improved the GF core performance indicator for reporting of number of people treated appropriately for malaria. By early 2013, Kenya was rated "adequate" (i.e., B1 rating) for overall grant performance, which will ensure continued GF funding. Funding for facilitated supervision should be included in GF plans.

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SEVERE NEUTROPENIA IN DENGUE: PREVALENCE AND SIGNIFICANCE

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Prolonged severe neutropenia unrelated to dengue is known to be associated with a higher risk for secondary infections. Peripheral neutropenia is commonly reported in dengue. However, its clinical significance is uncertain. We set out to determine the prevalence and duration of severe neutropenia in adult patients with dengue, and to investigate factors associated with severe neutropenia and its predictive capability for nosocomial infections or more severe clinical outcomes. We did a retrospective analysis of 1921 adult patients admitted to the Communicable Disease Center in Singapore between 2005 and 2008 with PCR confirmed dengue Severe neutropenia was defined as absolute neutrophil count ≤0.5x109/L, and dengue hemorrhagic fever (DHF) was defined according to the WHO criteria. The Kruskal-Wallis test was used to assess significance of continuous variables and x2 or Fisher's exact test for categorical variables, and logistic regression to identify independent factors associated with severe neutropenia. Our results showed that the prevalence of severe neutropenia is 11.8% (227/1921) with the lowest

neutrophil counts recorded on day 5 of illness. The median duration was 1 day. On the day of admission, only 2.4% had severe neutropenia. Age, Chinese ethnicity, inter-menstrual bleeding and hematocrit percentage were significantly associated with severe neutropenia in multivariate analysis. Severe neutropenia was not predictive for DHF, prolonged hospitalization stay or mortality. Our analyses also showed that severe neutropenia was not associated with a higher risk of secondary bacterial infections (pneumonia, urinary tract infection and *bacteremia*). In conclusion, severe neutropenia in adult dengue patients is frequent, but short-lived and not associated with nosocomial infections, prolonged hospitalization or increased mortality or DHF.

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POTENTIAL HARM OF PROPHYLACTIC PLATELET TRANSFUSION IN ADULT DENGUE

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Transient thrombocytopenia is common in dengue. Concern of bleeding risk from severe thrombocytopenia may lead to preventive platelet transfusion. Data from small series in neonatal dengue shock syndrome and adult dengue fever did not show benefit. We studied all hospitalized adult dengue patients at Communicable Disease Centre, Singapore from 2005 to 2008 with a positive dengue polymerase chain reaction or serology (fulfilled World Health Organization 1997/2009 probable dengue criteria) whose platelet was lower than 20x10^9/L without bleeding. We aim to study potential benefits and harms of preventive platelet transfusion. Of 6234 hospitalized patients, 809 developed platelet count ≤20x10^9/L without bleeding, and 498 were transfused platelet. At baseline, transfused patients had more leukopenia (3.4 vs. 3.9, x10^9/L), lymphopenia (28% vs. 31%), thrombocytopenia (14 vs. 16, x10^9/L), and neutrophilia (52% vs. 46%), and higher AST (208 vs. 153, U/L) and ALT (117 vs. 84, U/L) levels ($p<0.05$). The two groups were similar in age, fever duration, systolic blood pressure and serum hematocrit. While transfused patients had higher platelet increment the next day (8 vs. 5, x10^9/L), they had more mucosal bleeding (18% vs. 9%), longer time to platelet ≥50x10^9/L (3 vs. 2 days) and hospital stay (6 vs. 5 days), and more severe dengue (20% vs. 15%) ($p<0.05$). There was no difference in all clinical (23% vs. 18%) and severe bleeding (3.4% vs. 1.3%), dengue hemorrhagic fever (17% vs. 15%), intensive care admission (3.4% vs. 1.3%) or death (0.2% vs. 0%). Preventive platelet transfusion was associated with higher platelet increment but appeared to cause potential harm in prolonging platelet recovery and hospital stay without reducing all clinical or severe bleeding.

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EFFECTIVENESS OF ROTAVIRUS VACCINATION AGAINST SEVERE CHILDHOOD DIARRHEA - GUATEMALA, 2012

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Concerns remain about lower effectiveness and waning immunity of oral rotavirus vaccines in poor populations, where enteric co-infections, co-morbidities, malnutrition, and unusual rotavirus strains are common. We evaluated vaccine effectiveness against severe rotavirus disease in Guatemala, one of the first low-income countries to implement routine rotavirus vaccination in 2010. A case-control evaluation was conducted in inpatient and emergency department settings in 4 hospitals during 2012. Card-confirmed vaccine history was compared between case-patients (children with laboratory-confirmed severe rotavirus diarrhea) and 2 sets of controls: non-diarrhea hospital controls (matched by date of birth ±30 days) and rotavirus-negative diarrhea controls (adjusted for birth quarter). Vaccine effectiveness ((1-odds ratio of vaccination) x100%) was computed using logistic regression models. We enrolled 191 case-patients, 540 non-diarrhea controls, and 291 rotavirus-negative controls. Case-patients and controls were similar for breastfeeding, birthweight, maternal education, and socioeconomic variables. An uncommon G12P[8] strain, heterotypic to the vaccine strain, was identified in 90% of rotavirus cases. Effectiveness of a full vaccine series against severe rotavirus diarrhea was 66% (95% confidence interval [CI]: 37%-82%) with non-diarrhea controls, and 68% (CI: 41%-83%) with rotavirus-negative controls; partial vaccination (one dose) was 52% (CI: -85%-88%) and 61% (CI: 4%-84%) effective, respectively. No significant differences in effectiveness were observed between infants 6-11 months (83%; CI: 33-96) compared to children ≥12 months of age (64%; CI: 23-83) (P=0.3). Rotavirus vaccination provides protection through 2 years of life against severe rotavirus diarrhea caused by a heterotypic strain among Guatemalan children. This supports broader implementation of rotavirus vaccination in low-income countries where >90% of the half million annual global deaths from rotavirus occur.

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DISPARITIES EXIST IN THE PROVISION OF TRAVELERS' DIARRHEA SELF-TREATMENT TO CHILDREN

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Travelers' Diarrhea (TD) is the most common illness in travelers, including children. The CDC Yellow Book endorses the use of antibiotics for self-treatment of traveler's diarrhea (STTD) in children but does not address the issue of anti-motility agents directly. This study is a retrospective review of antibiotic and anti-diarrheal prescriptions to military beneficiary children, ages 0-17, at pre-travel visits during 2010, traveling for <120days. Of 1557 visits, civilian medical providers accounted for 48%. Prescribing patterns were assessed and compared based on practice setting, provider specialty, and patient age. In total, 43% were not prescribed STTD. Across all ages, military clinics were more likely to prescribe STTD than civilian counterparts (OR=2.1, 95%CI 1.7, 2.6). This effect was seen in each age group and was most pronounced for infants and toddlers (OR=3.6, 95% CI 1.8, 7.2) and early adolescents (OR= 4.2, 95% CI 2.5, 7.0). Among military clinic visits, there was marked variation by specialty in the likelihood of STTD. Pediatric Clinics (PC) when compared toNon-Pediatric Primary Care Clinics (NPPCC) were more likely to provide STTD (OR=4.8, 95% CI 3.2, 7.2). This effect was seen in each age group, and most pronounced in school aged children (OR=7.1, 95% CI 3.2, 15.8) and infants and toddlers (OR=5.9, 95% CI 1.8-19.3). PC and Specialty Travel Clinics (STC) were similar in their use of STTD (OR=1.2, 95% CI 0.9, 1.7), except for infants and toddlers in which case STC were less likely to offer TD therapy (OR 0.36, 95% CI, 0.2-0.8). Loperamide use was low among both PC and NPPCC until middle adolescence when pediatric providers were more likely to prescribe it (OR 4.3, 95%CI 1.5, 12.1). STC were more likely to utilize loperamide than PC (OR 3.6, 95%CI 2.4, 5.3), particularly in school age (OR 2.4, 95%CI 1.1, 5.3) and middle adolescents (OR 2.6, 95% CI 1.0, 6.5). Pediatric travelers often do not receive CDC recommended care for STTD. Significant differences in STTD practice exist based on patient age and provider specialty and military affiliation. Evidence based guidelines for STTD specific to children, provider education, and decision support tools are needed.

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INVASIVE *SALMONELLA* INFECTIONS IN AREAS OF HIGH AND LOW MALARIA TRANSMISSION INTENSITY IN TANZANIA

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Invasive salmonellosis is a major cause of childhood febrile illness and death across sub-Saharan Africa. The epidemiology of *Salmonella* Typhi and invasive nontyphoidal *Salmonella* (NTS) differs; *Salmonella* Typhi is often driven by environmental risk factors, while host-related factors such as HIV infection and malaria are associated with invasive NTS. We compared the prevalence of malaria and *bacteremia*, in particular invasive salmonellosis, among hospitalized febrile children aged 2 months to 13 years at 2 sites in Tanzania. Teule Hospital (TH) and Kilimanjaro Christian Medical Centre (KCMC) are located in areas of high and low malaria transmission intensity, respectively. Sites employed similar study protocols and participants were enrolled at TH from June 2006 through May 2007 and at KCMC from September 2007 through August 2008. Blood culture using BacT/ALERT, malaria microscopy with Giemsa-stained blood films, and HIV testing were performed. At TH, 3,639 children were enrolled compared to 467 at KCMC. Smear positive malaria was detected in 2,195 (60.3%) of 3,639 at TH and 11 (2.4%) of 460 at KCMC (p<0.001). Bacteremia was present in 336 (9.2%) of 3,639 at TH and 20 (4.3%) of 463 at KCMC (p<0.001). NTS was isolated in 162 (4.5%) of 3,639 children at TH and 1 (0.2%) of 463 at KCMC (p<0.001). *Salmonella* Typhi was isolated from 11 (0.3%) patients at TH and 6 (1.3%) at KCMC (p=0.008). With NTS excluded, the prevalence of *bacteremia* at TH was 5.0% and at KCMC 4.1% (p=0.391). HIV prevalence among enrollees at TH was 3.9% compared to 13.2% at KCMC (p<0.001). Where malaria transmission was intense, invasive NTS was common and *Salmonella* Typhi was uncommon, with the converse true where malaria transmission intensity was low. Bacteremia was more prevalent at TH than KCMC, but when NTS was excluded, there was no difference in proportions of bacteremic children between the sites. Invasive NTS and *Salmonella* Typhi may compete in as yet undetermined ways, and the interactions between these pathogens, the environment, and the host is a compelling area for future research.

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BACTEREMIA IN CHILDREN UNDER FIVE YEARS ADMITTED WITH NON-MALARIA FEBRILE ILLNESS AT JINJA CHILDREN'S HOSPITAL, UGANDA

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Acute febrile illnesses are the leading cause of hospital admissions among children under five years of age in Africa. Malaria, viral and invasive bacterial infections are the most common causes. Making a definitive diagnosis is challenging in resource limited settings, and most acute febrile illnesses are managed presumptively as malaria with often serious consequences. The objective of the study was to determine the prevalence, clinical features and spectrum of bacterial aetiologies of *bacteremia* among children under 5 years of age admitted to Jinja Children's Hospital with antimalarial treatment despite negative malaria slide. A total of 250 children under 5 years admitted with acute febrile illness, receiving antimalarial treatment despite a negative malaria smear were enrolled into the study. Clinical assessment was performed and blood collected for culture and complete blood count performed at the Makerere University Medical Microbiology Laboratory. A total of 15 blood samples (6%) were contaminated and excluded in the final analysis. Bacteria were present in 44/235 samples giving a prevalence of 18.7%. *Staphylococcus aureus* was the commonest isolate 41% (18/44) followed by non typhoidal *salmonella* 25% (11/44), pseudomonas aeruginosa 11% (5/44) and streptococcus pneumonia 9% (4/44). The common clinical features among children with *bacteremia* were temperature ≥ 37.5^{0}C (86%), cough (84%), vomiting (47%), weight loss during the illness (45%), diarrhea (44%) and history of fever for ≥ 2weeks (30%). Bacteremia was significantly associated with fever lasting 2weeks or more (OR = 1.53; 95% CI, 1.05-2.24), history of weight loss during the illness (OR = 2.70; 95% CI, 1.37-5.34) and total White Blood Count Cell (WBC) ≥ 15,000cells/ul (OR = 2.02; 95% CI, 1.04-3.92). History of weight loss during the illness was an independent predictor of *bacteremia* (OR = 2.5; 95% CI, 1.26-5.00). Clinicians should have a high index of suspicion for *bacteremia* in children under 5 years with negative malaria slide especially those with history of weight loss. The prevalence of *bacteremia* in children admitted with acute febrile illness was high. Commonest bacterial pathogens were staphylococcus aureus followed by non typhoidal *salmonella*. Weight loss during the illness was an independent predicator of *bacteremia*.

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FIRST YEAR FINDINGS FROM AN ACUTE FEBRILE ILLNESS SURVEILLANCE STUDY IN PUERTO RICO

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Dengue is endemic in Puerto Rico but little is known about its epidemiology in relation to other acute febrile illnesses (AFI). To study this, enhanced AFI surveillance was implemented at a large referral hospital in Ponce, Puerto Rico. Outpatients with fever or history of fever for ≤7 days were enrolled with informed consent and followed through their illness. Specimens including serum and nasopharyngeal swabs were collected and tested by PCR and immunodiagnostic methods as appropriate for *Leptospira* spp (Lepto), *Burkholderia pseudomallei (Burk)*, 5 enteroviruses (Entero), influenza A (Influ A), influenza B (Influ B), 12 other respiratory viruses including adenovirus, respiratory syncytial virus, metapneumovirus, and parainfluenza viruses 1-4, and 4 dengue viruses (DENV). 1,739 of 6,495 AFI patients seeking care were enrolled between May 2012-March 2013; 31.9% were hospitalized, 46.9% were female, and the median age was 12.7 years (range: 0-93 years). Pathogens were detected in 1,206 (69.4%) cases; 1 (0.1%) Lepto, 3 (0.2%) Burk, 39 (2.2%) Entero, 135 (7.7%) Influ A, 157 (9.0%) Influ B, 221 (12.7%), other respiratory viruses, and 651 (37.4%) DENV cases were identified. Almost all (99.0%) of the 417 DENV cases were DENV-1. Forty-two (2.4%) PCR positive co-infections were identified; 24 (57.1%) were DENV and a respiratory virus. Dengue patients were more likely to be admitted than other enrolled patients (OR 2.11, 95%CI 1.71-2.61) or influenza patients (OR 2.95, 95%CI 2.12-4.12); they were also slightly older than other patients (median age of 14.5 versus 9.9 years) but not influenza patients (14.5 versus 13.5 years). No pathogen-specific differences in gender were noted among infected patients. The majority of AFIs were caused by DENV, respiratory viruses,

and enteroviruses. Leptospirosis and melioidosis cases may be more focal and sporadic in nature requiring longer study in more than one site. There is some evidence that dengue cases may have more severe presentations when compared to other AFIs. Data for the complete first year will be analyzed and presented.

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META-ANALYSIS OF URINE HEME DIPSTICK DIAGNOSIS OF *SCHISTOSOMA HAEMATOBIUM* INFECTION, INCLUDING LOW-PREVALENCE AND PREVIOUSLY-TREATED POPULATIONS

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Urogenital schistosomiasis remains endemic in many areas of sub-Saharan Africa. Current control is based on drug administration, targeted either to school-age children or to high-risk communities at-large. Urine dipsticks for detection of hematuria offer an inexpensive means for estimating infection prevalence. However, their diagnostic performance has not been extensively evaluated after community treatment or in areas with continuing low prevalence. The objective of the present study was a meta-analysis of dipstick accuracy for *Schistosoma haematobium* infection in endemic regions, with special attention to areas where infection intensity or prevalence is low. Studies were identified by search of online databases and hand search of existing study archives. Eligible studies included population surveys, irrespective of date, location, or language, that compared dipstick diagnosis of *S. haematobium* infection to standard egg-count parasitology. For 95 included surveys, variation in dipstick sensitivity and specificity was evaluated according to study size, age- and sex-specific participation, region, local prevalence, treatment status, and other factors potentially affecting test performance. Independent of prevalence, greater accuracy was seen in surveys of school-age children, whereas performance was less good among surveys performed in North Africa. By hierarchical summary ROC analysis, overall dipstick sensitivity and specificity for detection of egg-positive urines were estimated at 81% and 89%, respectively. Sensitivity was lower among treated populations (72%) and in population subgroups having lower intensity infection (65%). When the insensitivity of egg counting was considered, and diagnosis was instead inferred from combined hematuria + egg count findings, overall dipstick sensitivity/specificity was 82%/97%, but with significantly better sensitivity (92%) in higher prevalence settings. This analysis suggests that dipsticks will continue to serve as useful adjuncts for monitoring community prevalence following implementation of urogenital schistosomiasis control.

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MICRO-GEOGRAPHICAL HETEROGENEITY IN *SCHISTOSOMA MANSONI* AND *S. HAEMATOBIUM* INFECTION AND MORBIDITY IN A CO-ENDEMIC COMMUNITY IN NORTHERN SENEGAL

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Schistosoma mansoni and *S. haematobium* are co-endemic in many areas in Africa. Yet, little is known about the micro-geographical distribution of these two infections or associated disease within such foci. Such knowledge could give important insights into the drivers of infection and disease and as such better tailor schistosomiasis control and elimination efforts. In a co-endemic farming community in northern Senegal (n=599), we studied the spatial distribution of *S. mansoni* and *S. haematobium* single and mixed infections (by microscopy), *S. mansoni*-specific hepatic fibrosis, *S. haematobium*-specific urinary tract morbidity (by ultrasound) and water contact behavior (by questionnaire). The Kulldorff's scan statistic was used to detect spatial clusters of infection and morbidity, adjusted for the spatial distribution of gender and age. *S. mansoni* and *S. haematobium* infection densities clustered in different sections of the community (p=0.002 and p=0.023, respectively). This divergent pattern was related to the use of different water contact sites. Furthermore, the *S. mansoni* infection cluster overlapped with that of severe hepatic fibrosis. Within that cluster, more severe hepatic fibrosis clustered in a small group of adults living adjacent to the most frequently used water contact site (RR=6.3; p=0.043). *Schistosoma* infection and associated disease showed important micro-geographical heterogeneities with divergent patterns for *S. mansoni* and *S. haematobium* in this Wolof community. Further in depth investigations are needed to confirm the micro-geographical segregation of *S. mansoni* and *S. haematobium* infection and the strong geospatial clustering of chronic disease in other settings and over time, and to explain these phenomena. Yet, the present study indicates that micro-geographical patterns should not be overlooked in schistosomiasis-related research and control, and are crucial for elimination efforts.

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HIGH *SCHISTOSOMA MANSONI* DISEASE BURDEN IN A RURAL DISTRICT OF ZAMBIA

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Schistosoma mansoni is endemic in most parts of rural Zambia, and complications such as hepatosplenomegaly, ascites and portal hypertension are commonly reported. We conducted a cross-sectional survey to determine the burden of *S. mansoni* disease and associated risk factors among 754 people (age range 7-50 years; mean 28.3) in four rural communities (Luampa, Mangango, Mwadasengo and Namando) of Kaoma district between September and October 2012. Kato-Katz technique was employed using duplicate stool smears to detect *S. mansoni* eggs. Intensity of infection was determined by obtaining average egg counts from two readings. Hepatosplenic disease was assessed using physical examinations and ultrasonography. The overall prevalence of *S. mansoni* infection and geometric mean egg count (GMEC) was 42.4% (304/717) and 86.6 epg (95% C.I 75.6, 99.6), respectively. Heterogeneity in disease distribution was observed within and among communities with various ecological patterns. Infection rates in Namando were 10 times more compared to Mangango (95% CI 5.20, 19.82; p<0.001) while Mwadasengo had high GMEC. High GMEC (119.2epg; 95% CI 78.9, 178) were observed in the age group 11- 14 years followed by the age group 15 - 18 years (113.4; 95% CI 79.0, 163). Although females had high infection rates than males (45.0% vs. 38.0%; p. value=0.069), higher GMEC (209.5epg vs. 191.2epg; p. value= 0.655) were recorded in males. *S. mansoni* /hookworm and *S. mansoni* /malaria co-infections were detected in 12.3% and 5.2% of the population, respectively while there was absolutely no *S. haematobium* detected.Ultrasonography detected hepatosplenic disease in 28 % (199) of the 710 participants examined, with the majority (87%) detected in individuals above 15 years of age. Severe hepatosplenic disease was detected in 42(6%) of the participants. The findings highlight high burden of *S. mansoni* disease in this area and calls for immediate interventions to avert complications associated with the disease.

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IMPACT OF TWO ROUNDS OF MASS DRUG ADMINISTRATION FOR SCHISTOSOMIASIS CONTROL IN WESTERN KENYA: COMPARISON BETWEEN COMMUNITY WIDE TREATMENT AND SCHOOL-BASED TREATMENT IN HIGH PREVALENCE AREAS - THE SCORE PROJECT

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There has been increased global commitment for schistosomiasis control through mass drug administration programs through either school based or community wide treatments. As part of the ongoing Schistosomiasis Consortium for Operational Research and Eliminaiton (SCORE) projects in western Kenya, we evaluated the effect of two rounds of Praziquantel on schistosomiasis prevalence and egg intensity and compared community wide treatment (CWT) where the whole community was treated, to school based treatment (SBT) where school age children were treated by health teachers in schools. Among 150 communities participating in the SCORE project in areas with >25% prevalence in western Kenya, data from 33 communities belonging to either CWT or SBT arms that have been surveyed three times so far is presented. Written informed consent and assent were obtained from parents/ legal guardians and minors respectively. Three consecutive stool samples were collected from 100 children aged 9-12 years in each school and two slides prepared from each stool. The Kato/Katz method was used to identify *Schistosoma mansoni, Trichuris trichura*, and *Ascaris* eggs in the fecal material. Data was analyzed using SPSS software and group means compared using ANOVA. The overall prevalence and egg intensity (eggs per gram, epg) in CWT before MDA followed by two rounds of MDA of *S. mansoni* were 56.8% (Range: 20-100), 127.41 epg; 45.4 % (Range: 14.3-90.3) 74.40 epg and 35.3% (Range: 8.6-84.8) 49.4 epg respectively. There was significant reduction in prevalence levels (P= 0.022) with no significant difference in egg intensity (P=0.99). In the SBT arm, the prevalence and egg density pre and post two rounds of MDA were 60.92% (Range: 59.66-62.17), 126.64 epg; 51.33% (Range: 50.8-53.4), 126.63 epg and 38.8% (Range: 10.2-83.7), 53.3 epg respectively. There was significant reduction in both prevalence and egg density after two rounds of MDA (P<0.01). Following two rounds of mass drug administration; there was significant effect in both prevalence and egg density in SBT while in CWT arm, the effect was only significant in the prevalence.

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CHALLENGES IN IMPLEMENTING COMMUNITY WIDE TREATMENT FOR THE CONTROL OF SCHISTOSOMIASIS IN WESTERN KENYA - THE SCORE PROJECT

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In areas with high prevalence for schistosomiasis, WHO recommends community-wide treatment (CWT) be provided rather than school-based treatment that is used in lower prevalence areas. We conducted CWT in 75 villages in western Kenya employing community health workers (CHW) as drug distributors. We compared CHW reported coverage with an independent household coverage survey to identify factors that may influence CHW reporting. Simple stratified random sampling was used to select 15 households in each village. A structured questionnaire was used to determine treatment coverage levels as well as levels of drug side effects experienced. A total 1125 households in 75 villages were visited. Up to 63.9% (37.8-94.1%) reported having been treated compared to the CHWs reported coverage of 86% (85.1-106.7%) in the same villages (P<0.0001). Only 20.6% reported being absent during the treatment with 51.7% reporting that the CHW did not visit their homes to offer treatment. Few people declined treatment for fear of side effects (0.65%). About 2.9% of the population surveyed reported having not heard about the project. Only 0.32% felt they were not sick hence didn't need drugs, while 0.55% reported they were pregnant and 0.08% were influenced by rumors. Up to 20.3% were children under 5 years. Of the total population surveyed, only 25.9% experienced side effects with abdominal pain being the most frequent complaint (46.0%), followed by diarrhea (31.7%). The significant difference between CHW-reported coverage and house-hold survey coverage exposed the challenges faced in the CWT strategy which include logistical problems in ensuring prompt assessment to allow for follow-up treatment where needed, early identification of village-specific factors that may influence coverage levels, management of community fears related to drug side effects and strategies for ensuring maximum coverage of school-age children at the community level.

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HIGH PREVALENCE OF *SCHISTOSOMA JAPONICUM* IN HUMANS AND BOVINES FROM NORTHERN SAMAR, THE PHILIPPINES

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Schistosoma japonicum is the causative agent of schistosomiasis in the Philippines, China and parts of Indonesia. In the Philippines, 6.7 million people live in endemic areas with 1.8 million having direct exposure through daily water contact activities. As a zoonosis *S. japonicum* infects over 40 mammalian species, including water buffalo which have been shown to be major reservoir hosts in China. In the Philippines, water buffalo (Carabao) have been considered unimportant hosts due to low prevalence and infection intensity found in previous studies. Six barangays from the Northern Samar municipality of Palapag were surveyed to determine the role of carabao and cattle in the Philippines Bovine samples were examined with an improved microscopy technique (formalin-ethyl acetate sedimentation (FEA-SD) and qPCR analysis, while human samples were examined by KK (Kato-Katz) in addition to qPCR. High *S. japonicum* prevalence was found in humans when using qPCR (90.36%), while KK showed a much lower prevalence (22.86%). High prevalence was also found in bovines when using FEA-SD (72.00%) and qPCR (81.50%). Intensity of infection varied for bovines with cattle having a higher epg (eggs per gram) (2.23) than carabao (1.49). The Bovine Contamination Index was calculated using the combined carabao and cattle epg (1.74) and showed that each bovine was excreting an average of 42,750 eggs into the environment daily. Bovines also had a high prevalence of *Fasciola gigantica* infection by both FEA-SD (95.00%) and qPCR (95.65%) techniques. Bovines, particularly carabao, due to their high rate of daily water contact, likely play a more substantial role in schistosomiasis transmission in the Philippines than has been reported previously.

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SPATIAL PREDICTION OF HUMAN *SCHISTOSOMA JAPONICUM* INFECTION IN THE PHILIPPINES: TOOLS TO SUPPORT DISEASE ELIMINATION

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Schistosoma japonicum infection is endemic in 28 of the 80 provinces of the Philippines and the most recent data on schistosomiasis prevalence has shown considerable variability within provinces. In order to increase the efficient allocation of parasitic disease control resources in the country, we aimed to describe the spatial variation in *S. japonicum* risk across the Philippines, quantify the role of the physical environment in driving the spatial variation of *S. japonicum* and develop a predictive risk map of *S. japonicum* infection. Data on *S. japonicum* infection from 35,754 individuals across the country were geolocated at the barangay-level and included in the analysis. The analysis was then stratified geographically for Luzon, the Visayas and Mindanao. Non-spatial multivariable models of *S. japonicum* prevalence were built, including age and sex of individuals and environmental variables (rainfall, land surface temperature and distance to inland water bodies) as predictors; residual spatial dependence in *S. japonicum* prevalence was investigated using semivariograms. Zero-inflated binomial (ZIB) Bayesian geostatistical models of *S. japonicum* prevalence were developed, and diagnostic uncertainty was incorporated.Results of the analysis show that in the three regions, males and individuals aged≥20 years had significantly higher prevalence of *S. japonicum* compared to females and children <5 years. The role of the variables of physical environment was different between regions of the Philippines. The geographical distribution of *S. japonicum* risk was widespread in the Visayas whereas in Luzon and Mindanao it was much more focal. This analysis reveals significant spatial variation in *S. japonicum* infection risk in the Philippines. This suggests that a spatially targeted approach to schistosomiasis interventions, including mass drug administration, is warranted. When financially possible, additional schistosomiasis surveys should be prioritized to high risk areas identified by our study in Luzon which are currently underrepresented in our database.

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IDENTIFICATION AND CLONING OF *TRIATOMA DIMIDIATA* IMMUNOGENIC SALIVARY PROTEINS TO DEVELOP TRIATOMINE EXPOSURE IMMUNOASSAYS

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Triatoma dimidiata is a vector of Chagas disease in sourthern Mexico. Current methods to assess triatomine infestation usually lack sensitivity and precision and also are time consuming and costly. New methodologies are required to monitor bug populations in endemic regions of Chagas disease. Saliva of hematophagous bugs contains proteins that can elicit an antibody response. This has been used as an epidemiological tool and biological marker of exposure to disease vectors. We used sera from four Balb/c mice exposed to *T. dimidiata* bites to evaluate antibodies against salivary proteins by Western Blot. Immunogenic salivary proteins of ~14, ~18 and ~79 kDa were recognized by all mouse sera. The 79 kDa protein may be an apyrase, which has cross reactivity with other arthropods such as *Aedes aegypti*. Besides, 14 and 18 kDa salivary proteins may be specific of triatomines and represent good candidates for molecular markers of exposure to *T. dimidiata* saliva. Therefore, based in sequences previously reported in Gene Bank we designed primers to amplify 14.6 and 18 kDa *T. dimidiata* proteins. Then we isolated mRNA from salivary gland of *T. dimidiata* bugs and we obtained the amplicons by RT-PCR. They were cloned in a pGex plasmid using a double digestion strategy with BamI and XhoI enzymes. Both plasmid will be expressed in a procariot system to obtaining recombinant proteins, those will be used to develop anti-*T. dimidiata* salivary proteins immunoassays to monitor vector exposure.

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GEOGRAPHIC DISTRIBUTION OF *TRIATOMA DIMIDIATA* (*REDUVIIDAE: TRIATOMINAE*) IN NORTHERN BELIZE

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The triatomine vectors responsible for transmission of *Trypanosoma cruzi*, the causative agent of Chagas disease, are widespread throughout much of Central and South America. As the influence of this neglected disease is continuously augmented by globalization, an understanding of disease epidemiology must include documentation of vector distribution across Chagas endemic regions. *Triatoma dimidiata* is the sole Chagas vector reported from Belize, yet literature pertaining to the local ecology and control of this vector has been scarce since initial reports from the 1960s. A recent study provided valuable information on vector population dynamics and distribution in southern Belize, yet vector distribution and infection rates in northern Belize remain unknown. Here, an initial report regarding vector distribution throughout northern Belize and localized infection rates of vector populations is provided. A brief comparison of collection methodologies is described and early recommendations for regional vector control and surveillance are presented.

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THE VECTOR MOSQUITO *AEDES AEGYPTI* AT THE MARGINS: SENSITIVITY OF A COUPLED NATURAL AND HUMAN SYSTEM TO CLIMATE CHANGE

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Dengue viruses circulate between mosquito vectors and humans, causing nearly 400-million dengue infections annually. In the last decade, the Americas have experienced a dramatic increase in severe disease cases (dengue hemorrhagic fever), with devastating public health consequences. Of particular concern is the potential for the expansion of intense dengue virus transmission into cooler, high altitude cities that are presently outside of transmission zones but may be at risk under scenarios of climate change, such as Mexico City. To address this problem we are employing a coupled natural and human systems approach to explore the ecology of *Aedes aegypti*, the mosquito vector of dengue viruses, in Mexico. A field study is being conducted along a transect from Veracruz City to Puebla City, ranging from relatively warm and wet low-elevation coastal environments with well established vector mosquito populations and intense dengue virus transmission, to comparatively cool and dry high-elevation mountainous areas which currently are free of the mosquito vector and local virus transmission. Along the transect we are measuring how climatic, socio-economic and infrastructure factors are coupled with *Ae. aegypti* abundance. These data are being synthesized into spatially and temporally predictive models to examine if, how, and why the range of the dengue vector *Ae. aegypti* may change in the future. Observational and modeling results from two field seasons will be presented, indicating strong linkages between climate and mosquito presence and abundance.

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INVESTIGATING VARIABILITY IN THE GUT MICROBIOTA OF THE DENGUE VECTOR *AEDES AEGYPTI*

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The *Aedes aegypti* mosquito gut microbiome has the capacity to dramatically alter the success of dengue virus infection in the mosquito midgut. Moreover, it has been shown that certain species of bacteria are much more effective at preventing the mosquito from becoming infected with the virus. Manipulation of the mosquito gut microbiota is therefore one potential approach to reduce disease transmission. In order to better inform control strategies that involve the mosquito microbiome, we are investigating factors that determine its size and composition. In the present work, we reared multiple field and laboratory strains of *Ae. aegypti* in a controlled laboratory environment. The field strains were collected from Singapore, Thailand and St. Kitts and have been raised in the laboratory for 6-8 generations since collection of the parental generation. The laboratory strains we used were Rockefeller (origin: Caribbean, ~1930s), Orlando (origin: Orlando, FL, ~1940s) and Waco (origin: Waco, TX, ~1987). To standardize exposure to environmental microbes, we mixed larval water between strains multiple times during larval and pupal development. We then assessed variability in the number and species distribution of culturable microbes in the midguts of sugar and bloodfed females from each strain. We found substantial variation between strains in the size of the midgut microbiome in sugar fed individuals and in response to bloodfeeding. Investigations into the molecular and genetic basis of strain-specific differences in the culturable gut microbiome and the impact on dengue virus infection will be discussed.

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TOWARD AN UNDERSTANDING OF HOST PREFERENCE IN *TRIATOMA SANGUISUGA* (*HEMIPTERA:REDUVIIDAE*)

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Although rare in occurrence, autochthonous transmission of *Trypanosoma cruzi*, the etiologic agent of Chagas disease, within the United States is an area of growing concern. The arthropod vector that has been most implicated in these cases is the Eastern Bloodsucking Conenose (*Triatoma sanguisuga*). In southeastern Louisiana, the prevalence of *T. cruzi* in the adult population of *T. sanguisuga* has been found to be near 60%. Given this high prevalence, it seems likely that ecological and behavioral characteristics of the vector prevent frequent transmission of the parasite to humans. However, little is known with respect to the natural ecology of *T. sanguisuga*, including whether it is closely associated with a specific mammalian host as has been found for other closely related *Triatoma* species in the southwestern United States. We undertook a multi-year, ecological study of the species to better understand its preferred blood meal sources. Because sylvatic rodents have been found to be the principal host for other *Triatoma* species, in order to test them for *T. cruzi,* a sample of rodents was taken from a known *T. sanguisuga* habitat. Over three years, a total of 59 rodents were collected from the same parcel of land and tested for infection by PCR for *T.cruzi* kDNA. To complement these collections, late instar and adult *T. sanguisuga* specimens were also collected from this area and are being analyzed by PCR for both *T.cruzi* infection and blood meal source. This analysis of > 500 specimens is underway and will be presented at the meeting. Preliminary results from an initial sample of thirty rodents suggest a relatively high prevalence (60%) of *T.*cruzi infection. The three species of rodents contained in this sample tested positive for *T. cruzi* at varying levels: *Neotoma floridana* (67%), *Peromyscus gossypinus* (58%), and *Mus musculus* (0%). These data will allow us to better understand the host feeding preferences of *T. sanguisuga* and therefore gain a more complete understanding of the sylvatic transmission cycle of *T. cruzi* in the southcentral United States.

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QUANTIFYING IMPACT OF MOSQUITOES ON QUALITY OF LIFE

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New Jersey, like many eastern states, has a persistent problem with the Asian tiger mosquito. This species and other mosquitoes reduce residents' quality of life through discomfort and possible risk of disease. To guide a comprehensive area-wide pest management project to control *Aedes* albopictus in two counties in New Jersey, we quantified the impact of mosquitoes on residents' quality of life. We interviewed residents of 121 randomly selected households in both counties between October and November 2010. We asked residents about their experience with mosquitoes in their neighborhood, the importance of mosquito control compared to other public services (1=not important, 5=extremely important), and rated residents' utility based on paired comparisons to known health states on the EuroQol scale from 0 (death) to 1 (perfect health). The majority (54.6%) of respondents considered mosquitoes to be a problem, rating its severity as moderate (30.6%), severe (12.4%), or extremely horrible (11.6%). Respondents reported an average (±SD) of 7.1±4.0 mosquito bites in a typical summer week. Mosquitoes prevented 59.5% of residents from enjoying their outdoor activities at least to some extent. Residents rated their mosquito experience during that summer on a scale of 100 (no mosquitoes) to 0 (mosquitoes invasion) at 56.7±28.7, and their overall utility at 0.87±0.03 comparable to being moderately anxious or depressed. Respondents rated the importance of enjoying porch and yard outdoors activities without mosquitoes (4.7±0.8) equal to that of neighborhood safety and higher than that of a clean neighborhood (4.6±0.9). In conclusion, these New Jersey residents report a 0.13 decrement in utility due to mosquitoes, possibly comparable to the decrement associated with depression, and rate mosquito control as an extremely important element of public service.

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GENETIC PEST MANAGEMENT AND SOCIETY: AN INTERDISCIPLINARY ASSESSMENT OF CURRENT AND EMERGING TECHNOLOGIES FOR DENGUE CONTROL

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Dengue fever has been receiving increased attention from scientists and health professionals as a neglected tropical disease escalating in prevalence throughout the globe. Given the complex nature of the dengue virus and its primary mosquito vector, *Aedes aegypti*, several methods involving genetically engineered mosquitoes are being researched as potential solutions to issues of dengue control. However, controversies surrounding the implementation of biotechnologies in the past suggest the need to pay greater attention to the social and cultural contexts in which these technologies interact. We bring an interdisciplinary perspective to an assessment of current and emerging pest management technologies for dengue, with particular attention to the broader social, cultural, economic,

and ecological settings wherein these technologies may be used. Based on our assessment, controlling the dengue virus and its primary vector will require a multifaceted approach. Control programs need to be assessed individually according to their specific context in order to be successful and sustainable. We survey multiple databases covering the fields of biology, communications, entomology, ecology, epidemiology, economics, policy, and genetics. To assist policy makers in deciding how best to ease the burden of dengue, we develop a decision tree that suggests actionable solutions to some of the complex and multifaceted issues of dengue control. Scientists, policymakers, non-government organizations, and the public are currently and will be involved in complex decisions with constantly changing variables. It is our hope that incorporating a contextually specific and interdisciplinary approach to dengue control programs will enhance the research being conducted and help inform policy decisions, thereby improving future outcomes for all involved in the process and all those affected by dengue each year.

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STATE-WIDE SCREENING OF *AMBLYOMMA AMERICANUM* FOR *EHRLICHIAE* AND SPOTTED FEVER GROUP *RICKETTSIAE* SPECIES IN VIRGINIA USING A NOVEL MULTIPLEX REAL-TIME PCR ASSAY

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The population of the lone star tick, *Amblyomma americanum*, has expanded in North America over the last several decades owing to expanding deer populations. *A. americanum* is known to be an aggressive and non-discriminatory biter and is by far the most common human-biting tick in Virginia. It is also a known carrier for several bacterial diseases, making it an increasingly important disease vector. Few studies of human pathogen prevalence in ticks have been conducted in the state of Virginia since the mid-twentieth century. With renewed interest in tick surveillance in Virginia, we undertook this study to survey *A. americanum* populations from around the state for the presence of three *Ehrlichia* species (*E. chaffeensis, E. ewingii,* and Panola Mountain *Ehrlichia*), and three spotted fever group *Rickettsiae* (SFGR) (*R. amblyommii*, *R. parkeri,* and *R. rickettsii*) using a novel six-plex real-time PCR assay. Our studies revealed a high prevalence (50-80%) of *R. amblyommii,* a non-pathogenic SFGR in all areas surveyed, along with a presence of all three *Ehrlichia* species (1-22%). *R. parkeri*, previously only known to be harbored within Virginia's *Amblyomma maculatum* ticks, was found in *A. americanum* in several surveyed areas within two regions with established *A. maculatum* populations. This suggests that within those two geographic regions, *A. americanum* and *A. maculatum* share one or more reservoir hosts. *R. rickettsii* was not found in any sample tested. Our study provides the first state-wide screening of *A. americanum* ticks in recent history and indicates that exposure to *R. amblyommii*, and to *Ehrlichia*e may be common. The high rate of *R. amblyommii* suggests serology may be misleading in clinical cases of tick-borne disease, and that *Ehrlichia* suspicion should be increased. These data may be of relevance to other regions where *A. americanum* is prevalent.

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CHARACTERIZATION OF RELATIONSHIPS AND DEMOGRAPHIC PARAMETERS OF FOUR *ANOPHELES PUNCTULATUS* SIBLING SPECIES OF PAPUA NEW GUINEA BY GENOME SEQUENCING

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Members of the *Anopheles punctulatus* (AP) group are the principal vectors of malaria and lymphatic filariasis in the Southwest Pacific. This group is comprised of 13 sibling species, five of which are considered to be major vectors in Papua New Guinea. Understanding species relationships and population diversity has important implications for the implementation of vector control programs. Unfortunately, very limited genetic data is available for AP mosquitoes, in part due to their evolutionary distance to *An. gambiae* and to the extensive divergence among AP sibling species, as reported previously. Here, we sequenced the genomes of four AP sibling species by shotgun sequencing and generated 74 to 340 million reads for each species. We *de novo* assembled each species' genome independently generating an average of 26,163 contigs (range: 14,407 – 41,925) with an average assembly size of ~151 Mb (146 - 161) and an N50 of 9,980 (4,664-16,229). We aligned the contigs from each genome to produce a total of 82,651,073 nucleotides aligned in all four species (representing ~30% of the genome) and sequenced at greater than 10 X coverage in each species. Using these aligned sequences and highly redundant next generation sequencing data from each species, we were able to identify several million fixed differences (positions variable between species) and DNA polymorphisms (variable within a species). This data allows us to i) rigorously examine the phylogenetic relationships among these sibling species, ii) query the extent of introgression between species and iii) characterize the effective population size and population dynamics of each species.Our findings will provide a framework for better understanding how alleles could be shared within and between species. With improved knowledge of each species ecology and evolution, our study can contribute to improve integrated mosquito management strategies and improve malaria and lymphatic filariasis elimination efforts in this part of the world.

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VECTORBASE COMMUNITY SUBMISSION SYSTEM

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VectorBase is a NIAID-funded Bioinformatic Resource Center that provides resources to investigate vectors of human pathogens. Currently we have the genomes of 11 vectors: *Anopheles gambiae, A. darlingi* and *A. stephensi* (malaria); *Aedes aegypti* (dengue and yellow fever); *Culex quinquefasciatus* (filariasis); *Rhodnius prolixus* and *Glossina morsitans* (trypanosomiasis); *Ixodes scapularis* (lyme disease); *Lutzomyia longipalpis* and *Phlebotomus papatasi* (leishmaniasis); and *Pediculus humanus* (louse-borne typhus, trench fever and louse-borne relapsing fever). In addition, for these and other vectors we have transcriptomes, proteomes, other "omics" data and, population data such as single-nucleotide polymorphisms (SNPs) and insecticide resistance phenotypes. In order to help scientists in the process of improvement or development of new strategies for controlling, or even eradicating vector borne diseases, as well as support basic science, VectorBase is committed to improving the system for community data submission, which has been divided in four sections as follows: 1. Gene annotation (models in GFF3 or fasta format) and metadata (gene name, symbol and description), which are meant to improve the organisms gene sets, through our Community Annotation Portal (CAP); 2. Gene transcript and protein data coming from colonized

or wild organisms, may be submitted for display on VectorBase using the expression browser and genome browser; 3. Linking genes to publications allows researchers to share their papers with the VectorBase community, making them visible on the VectorBase genome browser; 4. Phenotype data in the insecticide resistance database (IRbase), or variation data (*i.e.*, SNPs) in the population biology browser (PopBio). To submit, go to our community section at either our website home page or navigation tab, *www.vectorbase.org*. Attend this poster for an overview of the submission system, its ongoing developments, and discussion of suitability of this tool for the research community needs. Help or comments: *info@vectorbase.org*. Tutorials: *www.vectorbase.org/tutorials*.

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MATERIAL SCIENCE AND PARATRANSGENESIS: AN UPDATED APPROACH TO CONTROL TRANSMISSION OF LEISHMANIASIS BY SAND FLIES

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Leishmaniasis continues to be considered one of the most neglected tropical diseases in the world. No vaccines are currently available, and the best methods for control involve the use of chemical pesticides that not only carry environmental toxicity risk but also promote resistance in vector populations. Paratransgenesis, often referred to as a "Trojan Horse" approach to vector control, is an alternate approach to reduce vector competence by genetically manipulating symbionts commonly found in disease vectors. These symbionts are then reintroduced to the vector for colonization of the midgut and production of effector molecules that negatively interfere with pathogen development. A paratransgenesic platform has been tentatively developed for sand fly control and possible reduction or elimination of *Leishmania* transmission in endemic areas. In this study, we have engineered two bacterial species, *Pantoea agglomerans* and *Bacillus subtilis*, to constitutively secrete an inactive antimicrobial peptide molecule. These bacteria were fed to 2nd instar larvae of *Phlebotomus papatasi* utilizing a novel bioencapsulation method and the emerging adults were monitored for colonization of their midgut. Paratransgenic sand flies were allowed to feed while gut colonization was continuously monitored. Flies that remained colonized were mated to assess fitness (e.g., survival and fecundity of females) and the possibility of vertical transmission. We are also assessing direct effect(s) of feeding melittin to adult sand flies. Our results suggest that bioencapsulation can be effectively used to deliver genetically modified bacteria to sand flies, and explores the effects of mellitin on sand fly physiology.

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EMERGING PARASITE OR INCREASED AWARENESS? THE STORY OF *HYPODERMA TARANDI*

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The purpose of this report is raise awareness of clinical manifestations and diagnosis of myiasis caused by larvae of *Hypoderma tarandi*, a warble bot fly, widely distributed in the habitats of caribou and reindeer in Sub-Arctic regions of North America and Europe. We report the clinical history of 7 individuals including five children suffering from myiasis localized to the forehead and of additional three cases in which larvae was extracted from the eye globe, causing blindness in two patient. A diagnosis should be evoked in people seeking medical care in August -December due to recurrent migratory localized swellings of the forehead, enlargement of occipital lymph nodes with/without eye complaints. Such patients should be asked about recent trips (last summer-autumn) to Sub-Arctic regions. We wish to emphasize the importance of an integrated approach to diagnose this severe condition. Since the definitive confirmation by microscopy or genome sequencing requires extraction of a larva, meanwhile serological verification takes time to perform; we suggest that ivermectin should be given on clinical suspicion alone. We also wish to stress the importance of publishing case reports in the local medical press. Our first publication helped in the recognition of symptoms and in shortening diagnostic delay in quite a few patients including 14 children who were diagnosed in Northern Norway during autumn 2012. Diagnosed was confirmed serologically in all cases showing presence of antibodies against Hypodermin C, an enzyme indicative of *Hypoderma spp.* larva infestation.

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HIGH PROPORTION OF HUMAN BLOOD MEALS IN *TRIATOMA SANGUISUGA* INFECTED WITH *TRYPANOSOMA CRUZI* SUGGESTS POSSIBLE EPIDEMIOLOGICAL THREAT OF CHAGAS DISEASE IN LOUISIANA

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Trypanosoma cruzi, the causative agent of Chagas disease, is transmitted by hematophagous insects called triatomines or "kissing bugs". In 2007, the first autochthonous case of vectorial transmission of Chagas disease in Louisiana (the sixth in the US) was described. 1 *Triatoma sanguisuga* was the species suspected to be responsible for this transmission, but little is known about its ecology and behavior. We report for the first time the predominant vertebrate blood meal sources of this species. A sample of 49 *T. sanguisuga* collected at the location of the first described case of vectorial transmission in Louisiana was used for the study. The infection of the bugs with *T. cruzi* was determined by PCR using TCZ primers. 2 To determine the blood sources of individual bugs, we used a recently described assay, amplifying 12S rDNA from the abdominal content of the bugs using vertebrate universal primers, followed by cloning and sequencing of the amplicons. 3 The *T. cruzi* infection prevalence of the bugs was 55%. Blood sources were successfully determined for 43 of the 49 bugs. On average, the number of blood source species detected was 1.6/bug; the highest was blood from four different species detected in a same bug. Surprisingly, the American green tree frog was the predominant blood source found. This species had never been described before as a blood source for any species of triatomine. Human was the second most detected blood source: 48.8% of the bugs had fed on human, and 10 different human 12S haplotypes were found, showing that at least 10 people had been fed on. The other blood sources were raccoon, cow, dog, squirrel, cat and Eastern woodrat. Almost 40% of the bugs which fed on human were infected, suggesting the potential of Chagas disease transmission to human in Louisiana.

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ECOLOGY OF CUTANEOUS LEISHMANIASIS IN SINAI: LINKING PARASITES, VECTORS AND HOSTS

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Cutaneous leishmaniasis (CL) is a neglected clinical disease with high prevalence in northern and eastern parts of Egypt. Field investigations across the Sinai Peninsula from January 2005 to December 2011 revealed that only the zoonotic transmission cycle is widespread in this region, with 400-500 cases reported to clinics annually. CL is restricted to Northern Sinai districts along the northeastern border of Egypt. This study identified potential vectors and reservoirs involved in the regional transmission cycle. Three sand fly species (*Phlebotomus*) were detected: *P. papatasi*, *P. sergenti*, and *P. kazeruni*; of these, 0.41% of *P. papatasi* were found infected with *Leishmania*-like flagellates, but *P. sergenti* and *P.*

kazeruni showed no evidence of infection. In rodent populations, positive *Leishmania*-amastigote impression smears were recovered from *Gerbillus pyramidum* (14/31), *Gerbillus andersoni* (5/23), *Rattus rattus* (4/12). Although, this long-term study covered many districts of Sinai, only sand flies and rodents within El-Hassan, Rafah, and Beer Lehfen districts tested positive for infection. Restriction fragment length polymorphism revealed that only *L. major* was circulating in the area, with no evidence for the presence of *L. tropica*, except in five isolates from El Barth, Rafah, in 2005. Finally, an ecological niche modeling approach was used to test linkages between vector and pathogen species across the study region via analytical approaches and data streams that are completely independent of the field data.

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DENGUE SURVEILLANCE SYSTEM IN ACTION IN THE PHILIPPINES

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Dengue is the most important mosquito-borne viral disease in the tropics particularly in the Philippines. This study aims to present the country's dengue surveillance system in action and the status of dengue surveillance data. The roles of the Epidemic Surveillance and Response (ESR) and the Philippine Integrated Disease Surveillance and Response (PIDSR) of the Department of Health will be discussed. ESR is the country's innovative way to participate in the World Health Organization's challenge to reduce morbidity and mortality of dengue. PIDSR aims to harmonize all existing disease surveillance systems to strengthen the capacity of the local government units (LGUs). Surveillance system involves the stepwise monitoring of the spectrum of dengue illness and disease reporting from the smallest administrative division called barangay health centers (or Rural Health Units, RHUs) to district or provincial hospitals, to regional hospitals, and then to the National Epidemiology Center by respective advocates, coordinators and officers at different hierarchical levels with PIDSR training. Reported national dengue cases in 2008-2010 comprised 90.08% (249,883) admitted in hospitals, 4.15% (11,522) not admitted, and 5.76% (15,987) with unknown diagnosis. National ambulatory cases in 2008-2010 comprised 64.72% (1,249) in government hospitals, 30% (579) in private hospitals and clinics, and 5.34% (103) unknown. National hospitalized cases in 2008-2011 included 63.36% (255,577) in government hospitals, 32.62% (131,590) in private facilities, and 4.02% (16,200) unknown. Case fatality rate is highest among infants of less than 1-year old followed by 1- to 10-year old children. This study is relevant in understanding the burden of dengue, its control program, and in estimating its economic cost in the country.

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TEMPORAL VARIATION IN THE RELIABILITY OF PASSIVE SURVEILLANCE FOR ESTIMATING DENGUE INCIDENCE

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Dengue imposes a significant public health burden in tropical and subtropical regions. The full extent of its human and economic burden is not clearly defined, however, because surveillance systems routinely underrepresent total disease incidence. The extent of disease underreporting can be estimated by comparing case counts passively reported to local health authorities with those identified using more active and thorough methods, and calculating an expansion factor (EF). EFs can vary inter-annually, but observed variation in the accuracy of a single passive surveillance system for relative incidence over time has not been rigorously examined. We compared laboratory-confirmed dengue incidence in Iquitos, Peru, between tri-weekly door-to-door surveillance in longitudinal cohorts (study population approximately 4,300-9,000) with clinic-based surveillance in 11 health centers (intended to serve approximately 240,000 residents). Of the five years analyzed for this project, results from 2007-08 and 2008-09 demonstrate annual EFs of 3.1 and 4.9, respectively. Aggregate estimates, however, mask intra-annual seasonal variation that was as high as a weekly EF of 41.7 during the 2007-2008 peak in transmission. There was a positive linear relationship (0.077, 95% CI=[0.053-0.10], $p<0.001$) between cohort disease incidence and EFs over both years, so that as disease incidence increased, the accuracy with which clinic-based surveillance reflected this change decreased. Additional analyses assess the relationship between surveillance efficiency and epidemiologic factors such as circulating serotype and age stratification. Our results highlight the need for a clearer understanding of the relationship between passive surveillance data and disease incidence, which will better inform vector-control intervention decisions by public health authorities.

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EVALUATION OF A DENGUE COURSE FOR PHYSICIANS IN PUERTO RICO

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Prior to 2010, the clinical management of dengue patients by physicians in Puerto Rico was not consistent with World Health Organization guidelines, as evidenced by chart reviews of fatal dengue cases in 2007 and a physician survey in 2008. A four-hour, classroom style course on dengue clinical management was developed and given to 7,813 physicians in 2010; this course was required for maintenance of licensure in Puerto Rico. We evaluated course effectiveness by measuring differences in patient care practices before and after the course. We reviewed 430 adult and 1075 pediatric medical records at the 12 hospitals in Puerto Rico with the highest number of reported lab-confirmed dengue inpatients during 2008-9 (before the course) and 2011 (after). A mixed-effects logistic regression with a random effect for hospital to account for anticipated within-hospital correlation was used to compare selected indicators of dengue management. The percentage of patients who did not receive corticosteroids increased for adult patients from 30% to 68% (OR 5.6, 95% CI 3.2-10.0) and for pediatric patients from 91% to 96% (OR 2.7, 95% CI 1.3-5.6). Usage of only isotonic intravenous saline solutions increased for adult patients from 49% to 69% and for pediatric patients from 9% to 16% (common OR 2.3, 95% CI 1.6-3.2). Ordering of fluid input and output monitoring increased for pediatric patients from 26% to 39% (OR 2.0, 95% CI 1.4-2.8) and for adult patients from 19% to 22% (OR 1.1, 95% CI 0.6-2.0). A statistically significant improvement in the management of dengue inpatients between 2008-9 and 2011 was detected in the hospitals with the most dengue patients, an effect that was likely due to the 2010 dengue course. Despite these significant results, improvement in clinical management is still needed for steroid usage in adults, isotonic fluids in children, and fluid monitoring in both groups. An online version of the course has been developed which should expand its reach and sustainability.

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IDENTIFICATION AND GENERATION OF DENGUE VIRUS VACCINE CANDIDATES USING A VIRUS-LIKE PARTICLE PLATFORM

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Dengue viruses (DENV), which are members of the *Flaviviridae* family, comprise four distinct serotypes, called DENV-1, DENV-2, DENV-3, and DENV-4. Primary infection leads to lifelong protection against the infecting serotype, but subsequent infection by another serotype sometimes leads to potentially severe and life-threatening illness as a result of antibody-dependent enhancement (ADE) of infection by non-neutralizing, but cross-reactive, antibodies. Because non-neutralizing antibodies enhance pathogenesis, an ideal vaccine for DENV would direct the antibody response only to neutralizing epitopes, especially those shared by the four serotypes of DENV, but not non-neutralizing or partially neutralizing antibodies associated with ADE. We have developed a peptide display and affinity selection platform, which integrates epitope discovery capabilities of phage display with the high immunogenicity of virus-like particle (VLP) antigen presentation. We have constructed large (>10^{10}), diverse libraries of random peptides displayed on VLPs of the RNA bacteriophage MS2. We have shown that VLPs can be affinity-selected from these libraries using monoclonal antibodies (mAbs) and that selected VLPs can be used directly as immunogens to elicit epitope-specific responses. In this study, we performed affinity selections using a panel of broadly neutralizing anti-DENV monoclonal antibodies that recognize conformational epitopes in the EDIII or EDI/II domains of DENV E protein. These selections yielded a collection of VLPs displaying mimotopes of the viral epitopes and bound strongly to the selecting mAbs. VLPs were expressed, purified, and used to immunize mice. Collected sera will be assessed for neutralizing activity against all four DENV serotypes by a plaque reduction neutralization test using live virus or by dengue reporter virus particles. By targeting specific epitopes, our goal is to elicit specific high-titer responses against vulnerable domains of DENV, avoiding the complications associated with non-neutralizing and infection-enhancing antibody responses.

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AN UNRECOGNIZED OUTBREAK OF DENGUE - ST. CROIX, U.S. VIRGIN ISLANDS, 2012

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In November 2012, a school nurse in St. Croix reported 27 suspected dengue cases among 369 (7%) students and staff to the Virgin Islands Department of Health and CDC. An investigation was begun to estimate dengue virus (DENV) infection rates in schools island-wide. Six randomly selected public schools participated in a stratified two-stage cluster serosurvey. Case finding for suspected cases was conducted at St. Croix's sole hospital to identify patients who had dengue serologic testing from January 2012-January 2013. Stored specimens from December-January were obtained and tested further by real-time RT-PCR at CDC. A dengue knowledge and prevention practices survey was sent to a representative sample of the parents of students at six elementary schools. Of 168 student and 91 staff specimens, 19% of students (95% CI 13%-25%) and 12% of staff (95% CI 4%-20%) were IgM anti-DENV positive, indicating infection in the previous 3 months; 3 students and no staff were PCR positive, indicating current infection. Of the remaining 134 students and 78 staff who were IgM- and PCR-negative, 77% of students (95% CI 62%-91%, mean age 10.5 years) and 96% of staff (95% CI 91%-100%) were IgG anti-DENV positive, indicating past DENV infection. Hospital case finding identified 308 total suspected cases, of which 209 (68%) were from December-January; of these 209, 104 (50%) were positive by IgM or PCR. Of 715 parent surveys returned, 69% of parents in St. Croix (95% CI 66%-71%) could not identify the DENV mosquito vector, 41% (95% CI 39%-44%) did not know that these mosquitoes are peridomestic, and 51% (95% CI 48%-55%) did not use insect repellent. This large dengue outbreak infected nearly one in five students. Anti-DENV IgG prevalence indicated that most children in St. Croix have been infected by DENV. Despite the apparent high rate of DENV transmission among St. Croix residents, families were not well informed about prevention. In places where dengue surveillance is challenging, the utility of schools as sentinels for seasonal outbreaks should be investigated.

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CLINICAL AND LABORATORY RISK FACTORS OF DENGUE PATIENTS ADMITTED IN INTENSIVE CARE UNIT: A MATCHED CASE-CONTROL STUDY

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Dengue infection may result in severe clinical manifestations that required intensive care. However, there is limited knowledge on the clinical and laboratory risk factors of these dengue patients during first presentation at hospital. A retrospective hospital-based 1:4 matched case-control study was performed with 27 dengue patients admitted to the intensive care unit (ICU) from year 2004 to 2007, and 108 dengue patients who do not required intensive care matched by year of dengue presentation. Univariate and multivariate conditional logistic regression were performed. ICU dengue patients are significantly older (median age=44; P=0.003) and have diabetes (14.8%; P=0.031), compared to non-ICU dengue patients (median age=34; 2.8% diabetics). ICU dengue patients are presented in the hospital on median 3 days post fever (dpf), and progressed to DHF/DSS on median 4 days post presentation (dpp), and stayed in ICU for a median of 3 days. Among the 27 ICU dengue patients, there were 7 deaths that occurred on median 7 dpf and 3 dpp. The period of hospitalisation for ICU dengue patients is significantly longer (median 8 days;P <0.0001) as compared to non-ICU dengue patients (median 4 days). After adjusting for age and diabetes, haematocrit increased of more than 20% with thrombocytopenia (P=0.001), hypoproteinemia (P=0.037), signs of plasma leakage (P<0.001), hypotension for age (P=0.005), shock (P=0.006) and severe organ involvement (P=0.003) were found to be significantly more common in ICU dengue patients than non-ICU dengue patients. Furthermore, maximum pulse rate [adjusted conditional odds ratio(ACOR)=1.05;95% confidence interval(CI)=1.01,1.09], maximum neutrophils (ACOR=1.11;95%CI=1.04,1.17), urea (ACOR=1.26;95%CI=1.02,1.56) and creatinine level (ACOR=1.02;95%CI=1.01,1.04) were associated with higher risk of admission in ICU. These risk factors identified during first presentation may be useful in complementing the World Health Organization's severity classification of dengue patients that may further enhance clinical management of these high risk patients.

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UTILITY OF WARNING SIGNS IN PREDICTING SEVERE DENGUE AND GUIDING ADMISSION

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The recommendation from the 2009 World Health Organization guidelines for managing dengue is to hospitalize patients with any warning sign for observation and management. We evaluated the utility of using warning signs to guide hospital admission and predict disease progression in adults. We conducted a prospective cohort study from January 2010 to September 2012. Daily demographic, clinical and laboratory data were collected from adult dengue patients. Warning signs were recorded. The sensitivity, specificity, positive and negative predictive values of warning signs in guiding hospital admission and predicting disease progression were evaluated. Four hundred and ninety-nine patients with confirmed dengue were analyzed. The sensitivity of warning signs in guiding admission for dengue hemorrhagic fever (DHF) II-IV and severe dengue (SD) was high but specificity was 52% and 47.5%, respectively. Having any warning signs had 100% sensitivity in predicting progression to DHF II-IV and SD but specificity was 52% and 48%, respectively. Absence of any warning signs had a NPV of 91%, 100% and 100% for DHF I-IV, DHF II-IV and SD. Of those who progressed to severe illness, 16.3% had warning signs on the same day while 51.3% had warning signs the day before developing severe illness. Our findings demonstrated that patients without any warning signs can be managed safely in ambulatory care to reduce burden on healthcare resources. No single warning sign can independently predict disease progression. The window from onset of warning sign to severe illness in most cases was short.

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SELF-REPORTED PAIN INTENSITY USING THE NUMERIC REPORTING SCALE IN ADULT DENGUE MANAGEMENT

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Pain is a prominent feature of acute dengue as well as a clinical criterion in World Health Organization guidelines in diagnosing dengue. There is a paucity of data detailing the development of pain during acute dengue in different epidemiological groups and in relation to disease progression. We conducted a prospective cohort study in laboratory confirmed adult dengue patients managed at a tertiary infectious disease referral centre in Singapore using a self-reported 11-point Numeric Pain Scale to quantify and compare levels of pain during acute dengue between different age groups, gender, hospitalization status and dengue severity. Self-reported pain scores were measured at each daily clinic visit and analyzed using logistic regression. We also explored the use of pain score as an independent predictor of dengue severity. We found that 90% of patients reported pain during acute dengue with a trend towards greater reporting of moderate/severe pain in those below 55 years (68%) compared to those older (44%) though this did not reach statistical significance at the 5% level. We found no statistically significant differences in levels of pain reported by dengue patients stratifying by gender, hospitalization status, or disease severity. Highest pain scores were reported at days 5-7 of illness and diminished rapidly subsequently. Peak levels of pain did not reliably precede development of dengue hemorrhagic fever or severe dengue. Pain scores were not useful in predicting progression to severe disease.

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THE DENGUE VACCINE INITIATIVE PROJECT: PASSIVE FACILITY-BASED FEVER SURVEILLANCE IN CHILDREN AND ADULTS OF SANTA CRUZ COMUNA OF MEDELLIN, COLOMBIA, AND BANG PHAE DISTRICT OF RATCHABURI PROVINCE, THAILAND

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Dengue fever (DF) is a major public health problem in Colombia and Thailand. Colombia experienced its largest epidemic with almost 157,000 DF cases and 217 deaths in 2010; Thailand reported provincial incidence rates of 698/100,000 person-years. For further evaluation of the burden of dengue, the Dengue Vaccine Initiative (DVI) is conducting fever surveillance in adults and children in Medellin, Colombia and in Ratchaburi province, Thailand, likely early adopter countries of a dengue vaccine. DVI is conducting a passive facility-based surveillance complemented by a healthcare utilization survey (HUS) to determine the burden of DF in Bang Phae district of Ratchaburi province in Thailand and Santa Cruz comuna of Medellin in Colombia. In the surveillance, every other eligible febrile patients between 1-55 years-of-age have been enrolled. We collected acute and convalescent blood samples to test for DF using NS-1 rapid test, IgM/IgG ELISA, followed by RT-PCR. From the HUS, we identify the proportion of febrile cases missed by the passive surveillance. The 1st year of fever surveillances launched in Oct. and Nov., 2011 in Bang Phae Community Hospital (BPCH) and Santa Cruz Hospital (SCH), respectively. There were 42 DENV positive cases among 349 subjects in BPCH and 15 lab-confirmed cases among 147 subjects in SCH. From BPCH, 34 and 8 patients were diagnosed as secondary and primary DF, respectively. Among 42 ELISA-positive cases, 32 cases were positive on RT-PCR, showing that DENV2 is the most commonly circulating serotype. From SCH, 8 and 7 individuals were diagnosed as secondary and primary DF, respectively. RT-PCR results are being processed. Almost 30% of the individuals with recent fever reported to not seek care through our facilities. Thus, the incidence rates are 96.9/100,000 and 601/100,000 person-years for Santa Cruz and Bang Phae, respectively. More data will be available for presentation. he incidence rates for DF calculated were almost 100/100,000 and 600/100,000 person-years for Medellin and Ratchaburi, respectively. Epidemiologic data, in addition to other economic and private demand data collected, will be used as evidence for decision-making for dengue vaccine introduction in Thailand and Colombia.

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REGULATION OF THE ANTIOXIDANT DEFENSE IN MOSQUITO CELLS INFECTED BY DENGUE 2 VIRUS

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Dengue viruses (DVs) generally cause trivial deleterious effects in mosquito (C6/36) cells, in contrast to what occurs in mammalian cells. As superoxide was detected in C6/36 cells that had been infected with DV-2, suggesting that oxidative stress may occur in those cells. Nevertheless, antioxidant defense is actually elicited, leading to protection of the mosquito cells from DV infection. To understand the regulatory mechanism in association with elicited antioxidant genes, we have identified a novel *p53* from C6/36 cells infected by DV-2 for 24 h. Although the *p53* was classified as a tumor-suppressor gene in mammalian cells, we have recently proved that it has an alternative function involving in antioxidant defenses of DV-infected mosquito cells. Knockdown of the *p53* gene in DV-9 in infected

C6/36 cells with a specific synthetized dsRNA, both the superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) significantly increased at 48 hpi. Moreover, the cell death rate raised to 8.49% compared to those without knockdown of the *p53*-like gene. Among antioxidant genes tested in this study, catalase was identified to be specifically regulated by the *p53*-like gene. Furthermore, ROS concentration in DV-infected cells was shown to be involved in regulation on the cell death rate. It seems that catalase is one gene regulated by *p53*-like gene and involved in antioxidant defense of mosquito cells in response to DV infection, presumably dependent on reduced ROS accumulation.

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UPDATE ON THE DEVELOPMENT SANOFI PASTEUR RECOMBINANT CYD TETRAVALENT DENGUE VACCINE

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Results in 2012 from a Phase IIb efficacy trial of the CYD tetravalent dengue vaccine in the Ratchaburi province of Thailand, showed for the first time that a safe, efficacious vaccine against dengue is possible. It also raised questions on the reference PRNT assay methods, challenged some of the fundamental dengue vaccine development hypotheses, and served as a reminder of the complexity of dengue disease. Analyses have been carried out and are still ongoing to understand the main finding from the PhIIb study (that although efficacy was seen for 3 serotypes, none was seen for DENV2, despite satisfactory PRNT titers). These analyses included the sequencing of the clinical isolates from the PhIIb study, which, despite differences with the vaccine, were nevertheless cross neutralized in Vero cell-based *in vitro* assays In parallel, investigations continue to characterize vaccine-induced cellular responses: analyses from a trial in Singapore have confirmed the previous data showing the induction of broad serotype-specific Th1 responses dominated by IFNγ; in addition, ongoing long-term follow up shows that cellular reponses persist for at least one year after vaccination. Assessment of safety remains a critical component of the dengue vaccine program. As of January 2013, more than 28,900 have received one or more CYD-TDV vaccinations, with no safety signals identified in ongoing safety surveillance. A formal integrated safety analysis of 9 completed clinical trials with more than 5300 vaccinees confirms the good reactogenicity and safety profile seen in individual studies, including in the PhIIb study with 2 years of active follow-up. In the pivotal phase III efficacy trials, vaccinations have been completed and active surveillance is ongoing, and the drop out rate after 20 months is <5%. This low proportion attests to the considerable site preparation efforts by the local teams and the investigational teams' commitment, and illustrates the importance of dengue disease for the communities. The results of these phase III trials are expected by the end of 2014.

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SAFETY OF THE CYD DENGUE VACCINE: INSIGHTS FROM AN INTEGRATED ANALYSIS OF 5,344 INDIVIDUALS VACCINATED IN NINE CLINICAL TRIALS

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The live, attenuated CYD tetravalent dengue vaccine (TDV) is in phase 3 evaluation. We analyzed pooled safety data from all 9 trials completed to Aug. 2012 evaluating a 0-6-12 month schedule. In these trials 15433 CYD-TDV doses were received by 5344 participants, and 2175 received placebo. The overall SAE reporting rate was comparable in vaccines and placebo controls, and only 3 SAEs among vaccinees were considered as vaccine-related (headache [n=2], polymyalgia rheumatica). In all, 5 severe dengue cases have been documented: 2 among vaccinees and 3 among controls. Among adults, 12-17 year-olds and 2-11 year-olds, after any injection: solicited injection site reactions (SISR) were reported for respectively, 51.2%, 45.6%, 59.0% with CYD-TDV, and 16.8%, 32.4%, 48.5% with placebo, while solicited systemic reactions (SSR) were reported for 69.8%, 69.4%, 69.5% after CYD-TDV, and 44.5%, 59.5% and 57.2% after placebo, respectively. SISRs were reported at comparable rates after each dose and were most commonly injection site pain. SSRs were less frequent after 2nd and 3rd injections than after the 1st and were most commonly headache, malaise and myalgia. Solicited reactions were mild to moderate in almost all cases, resolving typically within 3 days. Unsolicited adverse events were injection site reactions, gastro-intestinal disorders and infections, and were assessed as vaccine-related for 7.2% of vaccines, and 2.8% of placebo controls. In this dataset, there was no increase of reactogenicity after successive doses, no marked difference in the safety profile between flavivirus seropositive and seronegative individuals at baseline, no serious allergic reactions, neurotropic or viscerotropic disease, and no excess of severe dengue cases in vaccinees compared to controls. All available clinical trial data therefore show CYD-TDV to have satisfactory safety profile, with no evidence of sensitization to severe dengue. Potential safety risks are carefully monitored in all ongoing trials, including a review by an Independent Data Monitoring Committee.

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LABORATORY INVESTIGATION ON RE-EMERGENCE OF DENGUE FEVER IN MOMBASA, 2013

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Since the last quarter of the 20th century, frequency of dengue epidemics has significantly increased in many tropical regions including several locations in Africa. The global incidence is currently estimated at 50-100 million cases reported annually from over 100 countries. In March 2013, samples from 2 febrile patients from a Mombasa hospital were sent to the Viral Hemorrhagic Fever laboratory at the Kenya Medical Research Institute/Walter Reed (KEMRI/WRP). Testing was conducted to rule out arboviruses or viral hemorrhagic fevers by ELISA and RT-PCR. Both samples tested positive for dengue virus by RT-PCR, specifically dengue serotypes 1 and 2 (DEN1, DEN2); however, they were negative by IgM ELISA. Passive surveillance was implemented at 7 local clinics/hospitals, which consisted of administration of a questionnaire to suspect cases including demographic and clinical data and collection of a blood sample. From March to April 2013 a total of 185 samples were collected. The samples were tested for exposure to dengue by IgM ELISA and RT-PCR at KEMRI/WRP and CDC laboratories in Nairobi, Kenya. The age range of the patients was 3-75 years. The majority of cases were male (54%). A total of 21/185 (11.3%) samples tested positive by IgM ELISA, while 60/185 (32.4%) were positive by RT-PCR. Serotyping results showed the following serotypes circulating in Mombasa, DEN1 33/60 (55%), DEN2 17/60 (28.3%), DEN3 4/60 (6.6%) and two samples had dual infections of DEN1 and 3. The current investigations have shown that multiple dengue viruses are circulating in Mombasa since the last confirmed dengue outbreak of 1982. Sequencing and phylogenetic analysis of the isolates will shed more information on the evolutionary patterns of the Mombasa dengue viruses.

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SEQUENCE ANALYSIS OF THE DENV2 STRAINS ISOLATED IN THE PHASE IIB CYD VACCINE EFFICACY TRIAL IN RATCHABURI, THAILAND

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A phase IIb clinical trial was conducted in the Ratchaburi province of Thailand with the tetravalent CYD vaccine. The observed lack of protection against DENV2 led to further exploratory analyses. We sequenced isolates from dengue cases on prM to E coding regions using PCR amplifications with serotype-specific primers designed to cover all known dengue strains. In all, 17 DENV1, 53 DENV2, 3 DENV3 and 4 DENV4 samples were sequenced. DENV1, 2 and 4 samples were each identified as belonging to single virus lineage. There were 3 DENV3 sequences in two distinct genotypes. Phylogenetic analyses show no relationship between efficacy, and the genetic distance between circulating and vaccine strains. Indeed, DENV3 and 4 genotypes differed from that of the vaccine but showed protection, in contrast to DENV2 for which the circulating virus and the vaccine' parental strain were of the same Asian I genotype. However, significant differences exist within this genotype, and the DENV2 strain circulating in Ratchaburi during the clinical trial had been described in 2010 in Vietnam as a new, and rapidly emerging strain, causing higher viremia in humans. One specific amino acid signature of that strain is E-83-226-228-346 (KKEY), which is different to the vaccine's parental DENV2 strain PUO 218. Mapping these amino acids on 3D models shows that the first 3 residues are close to each other on domain II, and easily accessible at the surface. Data in Genbank show that this specific profile consistently circulates in only three countries (Vietnam, Thailand, Cambodia) of continental South-East Asia. No such profile was identified in America, India, Carribean, Pacific, Africa or non-continental Asia. While no immune escape was seen for that lineage in Vero-cell based neutralization assays using vaccinee's sera, it cannot be excluded that circulating virus is able to overcome vaccine-induced immunity due to high *in vivo* fitness. Phase III efficacy trials in countries with and without this lineage of DENV2 will help understand the Thai phase IIb efficacy trial results.

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DENV REPORTER VIRUS PARTICLES AND THEIR UTILITY FOR CLINICAL AND FUNCTIONAL APPLICATIONS

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The lack of reliable, high-throughput tools for characterizing anti-dengue virus (DENV) antibodies in large numbers of serum samples has been an obstacle in understanding the impact of neutralizing antibodies on disease progression and vaccine efficacy. In the current study, we demonstrate the diagnostic utility of DENV RVPs for measuring neutralizing antibodies in human serum samples against all four DENV serotypes, with attention to the suitability of DENV RVPs for large-scale, long-term clinical studies. DENV RVPs are antigenically equivalent to live virus, show serotype-specific responses against human sera, and yield reproducible neutralization titers that are in statistical agreement with PRNT results. In addition, using a technology called Shotgun Mutagenesis, we created a comprehensive plasmid mutation library for DENV-3 prM/E, in which each amino acid was substituted. DENV RVPs containing each prM/E variant were produced and assayed for viral budding and infectivity in order to identify residues critical for infectivity. Critical residues were mapped and visualized on crystal structures for prM and Env. Taken together, DENV RVPs offer advantages for detecting immune responses with application to large-scale clinical studies of DENV and can be used for understanding important structure function relationships for DENV Env protein.

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THE DENGUE VACCINE INITIATIVE PROJECT IN COLOMBIA AND THAILAND: SERO-PREVALENCE STUDY IN CHILDREN AND ADULTS OF SANTA CRUZ COMUNA OF MEDELLIN AND BANG PHAE DISTRICT OF RATCHABURI PROVINCE

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Dengue infection is a major public health problem in both Colombia and Thailand, likely early adopters of dengue vaccines. Colombia experienced its largest epidemic with almost 157,000 DF cases and 217 deaths in 2010 and Thailand has reported provincial incidence rate up to 698/100,000 person-years. Often sero-prevalence data are not available and estimation of the overall disease burden is incomplete due to inaccurate capture of asymptomatic dengue infection cases in both adults and children. In preparation for the upcoming dengue vaccine, the Dengue Vaccine Initiative is conducting sero-prevalence study, linked to fever surveillance, in Colombia and Thailand. The Dengue Vaccine Initiative Project (DVI) is conducting a sero-prevalence study to determine the burden of inapparent dengue infection in Bang Phae district of Ratchaburi province in Thailand and Santa Cruz comuna of Medellin in Colombia. From randomly selected residents between 1-55 years-of-age in the catchment area population, we collect 2000 paired sera with 6 months interval to estimate age-specific sero-conversion rate. To evaluate dengue immunity status, we perform PRNT on those samples that show rise in the IgG ELISA. In the 1st year of the sero-prevalence study, 2012 and 2009 subjects were recruited in Bang Phae district and Santa Cruz comuna, respectively. As of April 2013, the in-house IgG ELISA testing is complete for the paired sera collected from Bang Phae and ELISA/PRNT data will be available for presentation at the conference. From 2009 paired samples collected from Santa Cruz comuna, the overall sero-conversion rate of 5.1% was found across the age-group. The highest sero-conversion rate (7.0%) was found among the children with 5-9 years of age, followed by those 35-44 years-of-age with 6.0% sero-conversion rate. From preliminary PRNT performed on a subset of sero-converted samples (n=118), only 1 sample showed monovalent response to DENV 4. Almost 74% (n=87) showed immune response against all 4 serotypes. The sero-prevalence data generated will complement clinical data from the fever surveillance, as well as other economic, behavioral, private demand data, to provide essential evidence for decision-making for vaccine introduction in Thailand and Colombia.

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DETECTION OF NS1 PROTEIN IN ACUTE PHASE SERUM SAMPLES IS NOT ENOUGH TO MAKE THE DIAGNOSIS OF DENGUE-4 INFECTIONS IN BRAZIL

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Dengue is an acute febrile illness resulting from the infection by any of the four dengue virus serotypes and affecting about 100,000 people annually worldwide. Ribeirão Preto city, in Brazil, has experienced several large dengue outbreaks. Due to this fact, a virus surveillance study was implemented to determine the serotypes circulating in the region and to start control measures as soon as possible. With this approach, we have been able to map the dengue serotypes circulating each year and to predict their alternance in causing the outbreaks. As an example, we detected that dengue-1 virus (DENV-1) was the predominant circulating serotype for the last three years but dengue-4 virus (DENV-4) had started

to circulate in the city late in the last year. The virus surveillance approach consists of sending to our lab about 20 acute phase samples per week, equally distributed from the city and randomly selected from city reference health centers. Samples were collected from patients with dengue-like symptoms. In the lab, samples were tested by NS1-ELISA, IgG- and IgM-capture ELISAs, according to manufacturer's instructions (PanBio, Queensland, AU), and then, by polymerase chain reaction (PCR). From late December of last year to early April of 2013, 223 samples were sent to the lab. Evaluation by the NS1-ELISA showed that 71 samples were positive, 18 were inconclusive, and 134 were negative. PCR results showed that out of 71 NS1-positive samples, 56 were positive for DENV-4 and 15 for DENV-1, and among the inconclusive samples, 15 were DENV-4-positive and one was positive for DENV-1. However, an interesting finding was that when analyzing the 134 NS1-negative samples, 72 were positive for DENV-4 and none for other dengue serotypes. It was clear that the NS1-ELISA was not adequately detecting the DENV-4 NS1 protein and one of the reasons for this finding was that most infections occurring in the city were secondary infections, resulting in the observed low-level NS1 detection. However, among those 134 NS1-negative samples, 94% of them were IgG-negative, showing that most of patients from whom the samples were collected were having primary infections. Thus, our results show that DENV-4 NS1 protein has not been adequately detected by commercial tests resulting in inadequate diagnosis of dengue-4 infections.

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INHIBITION OF CHOLESTEROL SYNTHESIS AND ITS EFFECT ON DIFFERENT PHASES OF DENV REPLICATION CYCLE

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Although antibody dependent enhancement (ADE) effect and immunology related factors have been appointed as causative for severe Dengue, several cases are not consistent with this hypothesis, suggesting that other metabolic molecules could be involved. It has been reported that cholesterol levels are regulated by DENV infection *in vitro* and that lipids are necessary for virus replication. In addition, Lovastatin (LOV) treatment reduces viral RNA on a DENV replicon system. The aim of this work is to evaluate the antiviral profile of three statins against DENV at different phases of the viral replication cycle.HuH-7 Hepatoma cells were infected with DENV at a MOI of 4. Cells were pretreated 6h pre-infection or treated 12h post-infection with LOV (5μM), Pravastatin (PRA) (5μM-10μM)) or Atorvastatin (ATO)(5μM). On a subset of cells, statin concentration was maintained by applying treatment every 12h. All cells were collected 48h post infection. Viral effect was measured by cytopathic effects detected and viral titter was measured by using the Plaque formation unit (PFU) assay in BHK-21 cells. A reduction in DENV infective particles was observed on all statin treated cells compared with the untreated control. DENV inhibition percentages for LOV, PRA and ATO were 50%, 20% and 70% respectively. 6h pre-treatment with statins slightly reduced virus titter while 12h post-infection continuous treatment result on a marked reduction on DENV infective particles up to 30% for PRA, 90% for LOV and 100% for ATO. A 12h post-infection treatment reduced viral titter more than the single pre-treatment dose but did not reach the inhibition levels of the post-infection continuous treatment. Our results indicate that inhibition of *de novo* synthesis of intra-cellular cholesterol results on reduction of DENV infectious particles and increased cytopathic effect. Furthermore, a higher inhibitory effect was observed when treatment was applied on latter stages of the replication cycle (post-infection single dose) and even more when treatment was maintained (post-infection continuous treatment). ATO showed the highest antiviral effect, which can be due to its larger half-life (14h). On the other hand, PRA showed the lowest effect, which can be related to its low lyposolubility.

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COMPREHENSIVE MUTAGENESIS OF PRM/E TO IDENTIFY AND CHARACTERIZE EPITOPES ON DENGUE VIRUS

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Shotgun Mutagenesis technology was used to identify high-resolution epitope maps for dozens of human antibodies targeting the immunodominant envelope protein (prM/E) of Dengue virus (DENV). Comprehensive plasmid mutation libraries for DENV-3 and DENV-4 prM/E, comprised of over 2000 individual mutant clones, were created in which every prM/E residue was individually mutated to a defined substitution, expressed in human cells, and analyzed for its effect on antibody reactivity and viral infectivity. The neutralizing human anti-DENV monoclonal antibodies (MAbs) used in our studies were derived from infected patient B-cells and therefore represent a significant protective response of the human immune system. For each MAb, we identified amino acids on prM/E that are required for antibody binding, and these residues were mapped onto prM/E crystal structures to visualize and compare epitopes. Our goal is to map epitopes on DENV prM/E, determine their role in viral protection and pathogenesis, and how they relate to protein function. The binding kinetics of many of the MAbs has also been measured using biosensor binding to intact DENV virions. We expect that our work will help define the range of immunodominant structures on DENV prM/E and identify novel neutralizing antibody epitopes that can be used for improved therapeutics, diagnostics, and vaccine development.

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NEUTRALIZATION OF WILD-TYPE DENGUE VIRUS ISOLATES BY ANTIBODIES ELICITED AFTER IMMUNIZATION WITH A TETRAVALENT DENGUE VACCINE

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A Phase IIb efficacy trial (Clinicaltrials.gov NCT00842530) of the CYD tetravalent dengue vaccine in the Ratchaburi province of Thailand was recently completed. Results indicated efficacy against symptomatic dengue caused by dengue virus (DENV) serotypes 1, 3, and 4, but none against serotype 2, despite measurable neutralizing antibody (NAb) responses, as determined by a validated PRNT50 assay. We sought to investigate whether circulating DENV strains had escaped vaccine-induced neutralization responses. We assessed NAb responses by a PRNT50 assay against the vaccine parental strains and circulating strains isolated during the study. Seven (7) isolates were selected based on sequence and phylogenetic analysis, and successful in-vitro amplification: 2 DENV-1, 2 DENV-2, 2 DENV-3, and 1 DENV-4. The optimal virus working dilutions and days post-infection were assessed to ensure accurate assignment of NAb titers with the circulating strains. In accordance with the protocol, sera drawn 28 days post dose 3 (PD3) of 284 participants, and from 74 dengue cases occurring after this time point were tested for NAb responses against vaccine parental strains. Exploratory testing for NAb responses against circulating strains was performed using PD3 sera from 45/284 participants and confirmed dengue serotype cases. Similar NAb titers were seen against the vaccine parental strains and recent circulating DENVs from Thailand. There was no lack of response against circulating strains, and neutralization of DENV2 did not show a different pattern to that observed against the other serotypes. The PD3 sera drawn prior to infection were able to neutralize the circulating strains in the Vero cell based PRNT50 assay. Given these data, it does not appear that circulating DENV strains escaped neutralization as measured by PRNT50 assay, and therefore does

not explain the efficacy trial results. Investigations are ongoing into the apparent contradiction between presence of neutralizing responses against serotype 2 and lack of efficacy.

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ASSOCIATION OF POLYMORPHIC VARIANTS IN TNF-ALPHA, IL-6, RECEPTOR FCΓRIIA AND VITAMIN D GENES IN HONDURANS' WITH DENGUE INFECTION

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Dengue is an important problem of health public in tropical and sub-tropical countries. On the other hand, the response to dengue infection is influenced by the genetic background of the host. To evaluate the association of polymorphic variants in TNF-alpha 308, IL-6, Receptor FCγRII A and Vitamin D between individuals with different dengue disease presentation. The study was carried out in Tegucigalpa, Honduras, Central America. The study population consisted of 150 participant: 50 dengue fever (DF) and 50 dengue hemorrhagic fever (DHF) plus 50 asymptomatic controls (AC), all of them with proven dengue infection. The dengue classification was done following the WHO criteria. The selected single-nucleotide polymorphisms (SNPs) were carried out by a Real Time PCR methodology, using sequence specific primers, obtaining the allelic genotype for each investigated genetic marker. Comparisons between three population groups showed in DHF there was significantly more allelic frequency of TNFa-308 genotype GG (OR= 7.76, p= 0.0006) than in the AC, more over the frequency of this genotype is proportionally higher as disease severity progresses (AC: 67%, DF: 86% and DHF: 94%); genotype AA is absent in both DC and DHF. For FcgRIIA-131 H/R and IL-6-174 we observed no differences in the different population groups. As for the VDR-352, there is a higher frequency for the homozygous genotype TT(40%) in DF compare with DHF and AC (27 y 22% respectivetly), there is a tendendy for this polymorphism can be implied in protection to the infection severity for dengue virus for this genotype(TT) (OR=0.43, p=0.0597). These results provide evidence for the first time in Honduran population about the genetic susceptibility to the infection for dengue virus. However further studies are still necessary.

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DEFINING EARLY TRANSCRIPTIONAL SIGNATURES OF THE IMMUNE RESPONSE TO A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE (DENVAX) IN NON-HUMAN PRIMATES

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The mechanisms by which vaccines induce a strong and diverse immune response for generating long-term protective immunity remain largely unknown. High-throughput transcriptional profiling has the advantage of providing insight into the features of an early immune response to vaccination that cannot be observed using standard immunological assays. In this study, we sought to measure the early transcriptional responses in cynomolgus macaques following vaccination with the tetravalent dengue vaccine DENVax, by different methods and routes of vaccine administration. Six groups of animals were administered DENVax by different routes (intradermal or subcutaneous), methods (needle and syringe or PharmaJet injector), and dose (single or two injections on day 0). A control group received PBS. DENVax induced an early transcriptional response in non-human primates that peaked on day 3 post-immunization. The molecular signature associated with DENVax is characterized by networks of significantly up-regulated genes involved in interferon signaling (p=4.71E-09) and the antiviral response (p=5.08E-06), and is similar to that seen in humans vaccinated with other live attenuated viruses, including YF17D and live attenuated influenza virus. The magnitude of this early interferon response was greater following subcutaneous administration than intradermal; similarly, animals inoculated subcutaneously also had stronger humoral responses. Two injections on day 0 elicited a greater number of significant changes in gene expression than a single dose, but up-regulation of key genes involved in interferon signaling and apoptosis were observed with both dosing schemes. Method and route of DENVax administration resulted in subtle differences in gene expression over time. These data suggest that the strength of the early immune response to DENVax may play a role in the magnitude of the subsequent adaptive response.

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IDENTIFICATION OF FUNCTIONALLY CRITICAL REGIONS OF DENGUE VIRUS ENVELOPE PROTEIN

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Our current understanding of the role of envelope protein E in mediating Dengue virus (DENV) infectivity is supported by structures of E in different infective states, yet we lack a detailed mechanism for the final stages of infection, where low pH triggers E structural transformations and interactions to promote virus-host membrane fusion. By generating reporter virus particles (RVPs) from a comprehensive DENV-3 prM/E mutation array so that each residue in the polyprotein is individually mutated and tested for ability to mediate fusion, we have identified key residues in E that are critical for viral infectivity. The locations and interactions of these critical residues explain how DENV E functions on an atomic level. These residues fall into 5 distinct functional groups that are proposed to: (1) provide fusion loop protection prior to triggering or post-triggering fusion loop support to stabilize the E fusion trimer, (2) enable hinge movements that occur between the E domain interfaces during structural transformations, (3) mediate the formation and triggered disruption of interactions between E and protein M, and ultimately enable "zipper" contacts with E stem region to drive membrane fusion, (4) facilitate formation and triggered disruption of contacts between E ectodomain and stem, and enable crucial zippering interactions that promote virus-host membrane fusion, or (5) provide a strong membrane anchor for the fusogenic E trimer, by means of cross-helix interactions between transmembrane regions E-T1 and E-T2. Our studies reveal novel details of the DENV fusion pathway by identifying molecular interactions and roles in structural transitions for key functional residues whose actions are critical for E dimer stability, the dynamic processes of pre-fusion structural triggering, and fusion of virus and host membranes.

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THE CARIBBEAN CONNECTION AND THE RE-EMERGENCE OF DENGUE IN EUROPE

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Dengue is caused by 4 different but antigenically related viruses, DENV-1 to DENV-4, transmitted to humans through the bites of *Aedes* mosquitoes. The disease is endemic in 100 countries from Asia, America, Africa and Oceania. In Europe, in the past century, Dengue epidemics occurred in Greece and other Mediterranean countries were the incriminate vector

was *Ae. aegypti*. After that, it disappeared from Europe, but another competent vector, *Ae. albopictus*, was introduced in late 70s.In recent years, *Ae. aegypti* was reintroduced in south Russia and in Madeira island (Portugal). In 2010, dengue re-emerged in the French Riviera and Croatia, with small outbreaks. Two years later, in October 2012, a sustained and explosive epidemic appeared in Madeira Island. Both, 2010 and 2012 outbreaks were caused by DENV-1.Travellers from different European countries acquired the infection in the tropics and carried the virus to areas were the vector is present. One viremic traveler could introduce the virus and initiate an autochthonous transmission of dengue in non-endemic area. The objective of this study was to describe the phylogeny and phylogeography of the DENV-1 introduced in Europe in the recent outbreaks. We analyzed complete E sequence from imported dengue infections acquired by returning travelers from The Caribbean and Latin American countries. Phylogenetic analysis revealed that all DENV-1 strains belong to genotype V. The strains introduced in France and Madeira clustered within different South American lineage. In conclusion, our data suggest that 2 different introduction of American DENV-1 occurred in Europe and were the responsible for the outbreak observed in 2010 in France and the epidemic of 2012-13 in Madeira, island.

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ASSOCIATION OF POLYMORPHISMS IN FCΓRIIA AND DC-SIGN1 WITH THE CLINICAL PRESENTATION OF DENGUE INFECTION IN A MEXICAN POPULATION

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Dengue is the world's most prevalent vector-borne viral disease. The dengue virus causes a spectrum of illness including asymptomatic infection, a mild febrile illness, and in a small portion of cases dengue hemorrhagic fever or dengue shock syndrome. In the state of Morelos, Mexico, dengue infection is an increasing problem, with 488.2 reported cases per 100,000 habitants in 2008. Two SNPs, rs1801274 of FcγRIIa and rs4804803 of DC-SIGN1, have previously been associated with resistance or susceptibility to severe dengue infection in addition to other infectious diseases. Both of these polymorphisms are located in genes which code for receptors with important roles in dengue pathogenesis, and the relationship between these SNPs and clinical dengue infection in Mexican populations is unknown. In this study, real-time PCR was used to characterize the distribution of rs1801274 and rs4804803 in subjects with asymptomatic dengue infection (n=145), dengue without complications (n=64), and severe dengue (n=35) in Morelos. In contrast to previous studies, the arginine (G) variant of rs1801274 was associated with greater clinical severity of dengue. Homozygotes for the arginine variant were significantly more likely to present symptomatic (uncomplicated or severe) infection compared with asymptomatic infection (p=0.027). The frequency of the arginine allele was also significantly associated with symptomatic infection (p=0.026). The G variant of rs4804803 was found to be very rare in the population of study, with a frequency of only 5.17%, and was not significantly associated with the clinical presentation of dengue infection. Logistic and ordinal regression models relating the SNPs to severity of infection were also generated, accounting for covariates including primary or secondary infection and other environmental variables. These findings demonstrate the variability and complexity of factors involved in the development of severe dengue infection. Gene interactions may explain why the effects of the rs1801274 FcγRIIa polymorphism differ between populations.

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VIRAL KINETICS OF PRIMARY DENGUE VIRUS INFECTION IN NON-HUMAN PRIMATES: A SYSTEMATIC REVIEW AND INDIVIDUAL POOLED ANALYSIS

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Viremia kinetics directly influence the clinical course and transmission dynamics of dengue virus (DENV). Despite a handful of studies examining DENV viral kinetics *in vivo*, the majority of the within-host dynamics are unknown. Significant ethical barriers prevent experimental studies in humans, and as such, non-human primates have been used as a model system for DENV infection for decades. In the current work we identify papers with experimental DENV infection in non-human primates and employ survival analytic techniques to estimate the time to viremia and duration of viremia as well as use mixed-effects models to assess associations between these and serotype, inoculating dose, viremia assay, and species of primate. We estimate that the median time to viremia in rhesus macaques (the most numerous species used) ranges from 2.63 to 3.32 days for DENV-2 and -1, respectively and that the median duration ranges from 3.13 to 5.13 days for DENV-4 and -2, respectively. We find no significant differences between species of primate for either time to viremia or duration; the time to viremia for DENV-4 is significantly longer than for DENV-2 and duration of viremia is significantly shorter for DENV-4 than DENV-1 and -2. When viremia was assayed by reverse transcription PCR (RT-PCR), time to viremia was significantly shorter and duration of viremia was significantly longer than with plaque-forming assays. Finally, a significant negative relationship exists between inoculating dose of virus and duration of viremia. Knowledge of the within-host viral kinetics of DENV in non-human primates will aide in understanding the transmission dynamics of sylvatic DENV in populations of non-human primates, an issue of growing importance as dengue vaccines become available.

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EARLY TRANSCRIPTIONAL RESPONSES THAT CORRELATE WITH NEUTRALIZING ANTIBODY DEVELOPMENT IN DENGUE VACCINE RECIPIENTS

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Dengue virus (DENV) is the most common mosquito-born virus worldwide, causing an estimated 400 million infections annually. Neutralizing antibodies elicited after infection play an important role in protection from subsequent infection with the homologous serotype, and development of neutralizing antibodies has been the primary endpoint for evaluating the immunogenicity of candidate dengue vaccines. However, little is known about early host responses following vaccination that lead to development of neutralizing antibodies, or to differences in the titer of neutralizing antibodies among individuals. To characterize the acute host response to dengue vaccination and identify early correlates of adaptive immune responses we examined the genome-wide transcriptional response to a live, attenuated dengue vaccine, DEN3Δ30/31, the DENV-3 component in the TV-003 tetravalent dengue vaccine candidate developed by NIAID. We analyzed longitudinal whole blood RNA samples (n=165) collected prior to vaccination, 7 times during the next two weeks, and on days 21,

28, 42 and 180 from 10 DEN3Δ30/31 vaccinees and 4 placebo recipients. Significant increases in transcript abundance after vaccination were evident between days 5 and 14 in 9 vaccinees who developed positive neutralizing antibody titers; the most notable feature was a wave of interferon-stimulated gene (ISG) expression that peaked between days 6 and 12 (median, day 9). ISG transcript abundance on day 8 correlated with antibody titer (PRNT60) measured on day 42 post-vaccination (Spearman's rho = 0.73). The single, non-responding vaccinee did not develop an ISG expression response following vaccination. A second set of transcripts with peak expression between days 12 and 20 was enriched for genes associated with lymphocyte proliferation and activation (p<1E-10). These findings suggest that it is possible to identify early correlates of protective adaptive immune responses soon after dengue vaccination, and provide a pathway for further understanding the cellular and physiological events leading to development of neutralizing antibodies.

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PHYLOGEOGRAPHY AND MOLECULAR EPIDEMIOLOGY OF AN EPIDEMIC STRAIN OF DENV1 IN SRI LANKA

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In 2009, a severe dengue epidemic occurred in Sri Lanka that caused higher mortality and morbidity than any previously recorded epidemic in the country. In each subsequent year, dengue has continued to reach epidemic proportions, posing a major clinical burden to the population. The 2009 epidemic correlated with a shift in the predominant disease-causing dengue virus serotypes in Sri Lanka: prior to the epidemic, two serotypes, DENV2 and 3, were isolated from the majority of patients presenting with serious dengue disease, however, in 2009, a previously undetected DENV1 strain dominated as the major causative agent of dengue disease, and DENV1 has persisted as the dominant serotype in Sri Lanka. We amplified dengue virus from sera of patients who presented with severe disease to Colombo North Teaching Hospital in Sri Lanka during the Spring and Summer of 2012, and sequenced the full genomes of several DENV1 isolates. We report phylogenetic evidence that the 2009 epidemic DENV1 strain has continued to circulate within the population and was a causative agent of severe disease in Colombo, Sri Lanka during the 2012 epidemic. We applied bayseian phylogeographic methods to infer the historic spatial dispersion of this virus, using our Sri Lankan virus isolates and other reported sequences in the literature. These analyses suggest that the 2009 Sri Lankan epidemic DENV1 strain may have traveled directly or indirectly from Thailand, through China, to Sri Lanka, and, after spreading within the Sri Lankan population, traveled to Pakistan and Singapore. Our findings delineate the dissemination route of a virulent DENV1 strain in Asia and are of particular importance to global control efforts.

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CO-INFECTION WITH DENGUE AND RESPIRATORY VIRUSES AMONG CHILDREN WITH ACUTE FEBRILE ILLNESS, PUERTO RICO

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Dengue is endemic in Puerto Rico with seasonal increases in incidence that often co-inside with increases in other acute febrile illness (AFI) due to viral respiratory pathogens. This can lead to difficulties in clinical diagnosis and delayed recognition of dengue or these other AFIs. A sentinel enhanced dengue surveillance system site in a tertiary care hospital in Ponce, Puerto Rico began conducting AFI surveillance in May 2012. Outpatients with fever or history of fever for <7 days were enrolled with informed consent and followed through their illness. Specimens including serum and nasopharyngeal swabs were collected and tested by RT-PCR and immunodiagnostic methods as appropriate for a number of pathogens including 4 dengue viruses (DENV-1-4), influenza A, influenza B, and 12 other respiratory viruses including adenovirus, respiratory syncytial virus, metapneumovirus, and parainfluenza viruses 1-4. From May 2012 through January 2013, 18 PCR positive co-infections with DENV and a respiratory virus were identified among the 1439 enrolled case-patients. Most (91%) case-patients with co-infections were children and adolescents <18 years of age. When compared with 398 DENV positive only case-patients, co-infected case-patient reported runny nose and cough 2.4 and 3.0 times more frequently, but this was not statistically significant. Nevertheless, chest x-rays were ordered 7 times more frequently in co-infected case-patients as compared to DENV only case-patients. No other symptom or sign, risk factor, or laboratory test was associated with co-infection. The overall prevalence of co-infections is low. Additional studies should be performed to evaluate outcomes associated with severity. However, in concurrent epidemics of dengue and respiratory pathogens, physicians must have a high index of suspicion of co-infection.

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VACCINATION OF NON-HUMAN PRIMATES WITH DENVAX ELICITS SEROTYPE-SPECIFIC CD4$^+$ AND CD8$^+$ T CELLS WITH A PROINFLAMMATORY CYTOKINE PROFILE

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Dengue viruses have a significant impact on global health in the tropics and subtropics, particularly in Asia and Latin America. Vaccine development against dengue has accelerated in recent years with several candidate vaccines currently undergoing clinical trials. In this study we describe the characterization of T cell responses to DENVax, a chimeric dengue-2 PDK-53-based tetravalent vaccine in non-human primates. The vaccine was administered intradermally or subcutaneously with a needle and syringe or a needle-free device (PharmaJet) on day 0 and 60 and the kinetics of CD4$^+$ and CD8$^+$ T cell responses were monitored using flow cytometry. The vaccine was found to elicit T cell responses with both T cell subsets producing proinflammatory cytokines, IFN-γ, TNF-α, and IL-2 one month after priming. CD4$^+$ and CD8$^+$ T cells targeted both structural (E

proteins from each serotype) and non-structural proteins (NS proteins from DENV-2). Following challenge with wtDENV-2 or wtDENV-4, vaccinated animals were protected and frequencies of $CD4^+$ and $CD8^+$ T cells were elevated as compared to the sham immunized animals. These findings highlight the immunogenic profile of DENVax and suggest that dengue-specific T cell responses together with neutralizing antibodies might play a critical role in protection.

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USEFULNESS OF CLINICAL AND HEMATOLOGIC FINDINGS TO DISCRIMINATE DENGUE FROM OTHER FEBRILE ACUTE ILLNESS IN AN ENDEMIC COUNTRY

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Dengue is the most important arboviral infection in humans, whose frequency has increased in the world over the past 30 years. In tropical countries is essential to differentiate dengue from other infectious diseases. We assembled a cohort of febrile patients recruited from 2003 to 2011, during an epidemic and a non-epidemic dengue period in Colombia. Patients underwent clinical evaluation and hematologic testing within 3 days after the onset of fever. Dengue confirmed cases (DCC) had to have an IgM seroconversion from acute to convalescence samples, a fourfold increase in IgM titers or a positive virologic test (RT-PCR, viral isolation or NS1). Patients not meeting these criteria were referred to as non-dengue cases (NDC). We used multiple logistic regression to determine the contribution of predictors to the likelihood of DCC. We evaluated 1,476 febrile patients (49% DCC, mean age: 22.9 years). Patients with respiratory symptoms - rhinorrhea and cough - were 49% less likely to be DCCs (OR=0.51, 95%CI: 0.40-0.66). Other symptoms such as vomiting (OR=1.43, 95% confidence interval [95%CI]: 1.12-1.81), exanthema (OR=1.52, 95%CI: 1.19-1.94), and clinical findings including somnolence (OR=1.82, 95%CI: 1.30-2.55), body temperature (OR=1.18 per 1°C, 95%CI: 1.05-1.43), and orthostatic hypotension (OR=1.64, 95%CI: 1.13-2.37) increased the probability of DCC as well as a lower platelet count (OR=1.05 per 10,000 platelets/mL reduction, 95%CI: 1.03-1.06). There was an attenuation of the association between leukocyte count and the probability of DCC during the epidemic as compared to the non-epidemic period (OR=1.26 vs.1.44 per 1,000 cells/mL reduction, p=0.036). The model adequately fit the data with an area under de ROC curve of 0.77 (95%CI: 0.75-0.80). The combination of simple clinical findings and easily accessible hematologic tests could help physicians to discriminate dengue from other febrile illnesses in primary health care settings. These findings might be instrumental to developing a diagnosis algorithm in dengue endemic areas.

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EVALUATION OF THE CLINICAL AND LABORATORY DIAGNOSIS IN PATIENTS WITH SUSPECTED DENGUE

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Dengue fever (DF) is a disease with nonspecific symptoms and a broad clinical spectrum. Laboratory confirmation, using specific serological and virological tests, is essential for a conclusive diagnosis of dengue and to distinguish dengue-like diseases (DLD). Thus, the aim of this study was to evaluate the laboratory data of patients with clinical suspicion of dengue. Patients with acute febrile syndrome were recruited during the epidemic season of 2012 in the State of Ceará, Brazil. Clinical data and blood samples were collected in febrile (< 7 days) and convalescent (≥ 7 days) phases. The following laboratory tests were performed: immunochromatographic NS1 (Bio-Rad®), IgM ELISA (PanBio®), RT-PCR (Qiagen OneStep®) with primers AD3 and AD4, viral isolation (VI) and nonspecific tests (NT). We recruited 88 patients with clinical suspected DF. Nine patients (10%) had a positive NS1 and one (1%) was positive by RT-PCR reaction. Only 44 patients collected convalescent serum samples and 33 of them had a positive IgM test (75%). Of the 50 samples who had VI fulfilled, 3 (6%) were positive for serotype 4 (DEN-4). Thus, 38 patients have been confirmed diagnosis of dengue by at least one of the tests. Regarding symptoms, none analyzed showed significant differences between groups. The most common symptoms for both were: headache, retro-orbital pain, myalgia, arthralgia and prostration. The analysis of NT was significant different only the number of platelets ($p = 0.0086$), being the average of the DF group was 108.000/mm3 and the DLD group 146.000/mm^3. Thus, 48 patients remained without confirmed diagnosis, which may probably mean a failure of diagnostic tools or represent another dengue-like disease. We observed a lower than expected sensibility of virological methods, possibly associated with DEN-4 circulation. Thus, our data suggest the need to improve the accuracy of virological methods to allow the correct diagnosis of dengue and to discover new etiologies of DLD.

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DENGUE RISK AND PREVENTION IN EPIDEMIC-PRONE RIBEIRAO PRETO, BRAZIL: ANALYSIS OF KNOWLEDGE, ATTITUDES AND PRACTICES

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As many as 100 million cases of dengue occur worldwide each year. Diverse community-level and environmental risk factors for dengue virus transmission include urbanization, population density, climate, personal behaviors, and more. Although dengue risk is both socially and environmentally defined, the role of people's knowledge, attitudes and practices (KAP) concerning disease risk and prevention is not well understood. Accordingly, we studied the KAP of people regarding health-related decisions and behaviors intended to reduce dengue disease in urban Ribeirao Preto, Brazil. A KAP questionnaire was developed with and administered by trained Vector Control Agents of the Municipal Secretariat of Health of Ribeirao Preto in October and November 2012. Adults visiting any of 24 supermarkets in the city were asked to respond to a structured questionnaire. Each supermarket was selected according to vehicular traffic patterns, accessibility, geographic location, and consumer volume to achieve a representative sample. A total of 2,150 adults who consented to participate answered a series of 10 basic sociodemographic questions and 41 open- and closed-ended dengue KAP questions that involved vector ecology, viral transmission, clinical manifestations of the disease, as well as people's attitudes toward disease prevention responsibilities. Results suggest that people possessed adequate knowledge of the disease, especially in terms of recognizing breeding sites, transmission, and basic symptomatology. Dengue was not considered to be a disease of individual-level poverty, rather was thought to be related to neighborhood characteristics. Participants tended to view prevention as a shared responsibility of individuals, neighborhoods, and governmental agencies. Implications of these findings are evaluated in the context of historical patterns of intense transmission in Ribeirao Preto during the past decade.

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RISK FACTORS FOR DIARRHEA-ASSOCIATED DEATH AMONG CHILDREN IN BOTSWANA IN 2012

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Diarrhea is a leading cause of child mortality in Botswana, a country with high prevalence of childhood malnutrition (~11%) and adult HIV (~25%). For HIV-exposed infants (born to an HIV-infected mother), formula feeding is recommended. A two-fold increase in reported nationwide childhood diarrheal deaths between January-June 2012 prompted an investigation of risk factors to improve prevention and control efforts. A case-control study was conducted at main referral hospitals of 5 districts with the highest diarrhea case-fatality rates. Case-patients (children <5 years who died with gastroenteritis between January 1-June 30, 2012) were compared to age frequency-matched controls (children presenting to surrounding child welfare clinics [CWC]). CWC cards were reviewed for information on malnutrition (weight-for-age z-score <-2), HIV exposure, and exclusive breastfeeding through 6 months of life. Bivariate and multivariate logistic regression were performed to identify risk factors. Sixty-three case-patients and 126 controls were enrolled. Among case-patients, 34 (54%) were male, median age at death was 4 months (range: 0-18 months), 65% had severe dehydration on presentation, and 90% received intravenous fluids. Compared to controls, children who died were more likely to be HIV-exposed (97% versus 34%; adjusted odds ratio [aOR]=32; 95% confidence interval [CI]: 2-530), not exclusively breastfed (94% versus 28%; aOR=13; 95% CI: 2-84), and malnourished (44% versus 2%; aOR=42; 95% CI: 4-427). The vast majority of diarrhea deaths in Botswana occur among HIV-exposed infants who were not exclusively breastfed. Our findings support WHO recommendations to promote breastfeeding regardless of HIV exposure status and for effective treatment of malnutrition as key childhood survival interventions, and could contribute to efforts in Botswana to develop similar policies.

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BCG NANOEMULSION AS ADJUVANT AND VACCINE DELIVERY SYSTEM

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The development of effective vaccines is essential for controlling disease. Recombinant vaccines are widely used due to its ease to produce and dose adjusts. Unfortunately, recombinant vaccines enjoy low immunogenicity, often requiring the help of adjuvants. Here, we present the development of a novel and potentially effective adjuvant. Inactivated *Mycobacterium bovis* Bacilli Calmette-Guerin (BCG) is used as an active component in the complete Freund adjuvant. Cell wall components (BCG-CWCs) are the most immune-stimulatory part of BCG. Recently, nanotechnology has become integral to medicine and medical research, especially in the areas of drug and vaccine delivery systems. Nanoemulsions (NEs), emulsions with droplet-size in nanometer scale, have been shown to improve the antigenicity of weak antigens as it can easily be up-taken by phagocytes and freely circulate through lymphatic vessels. In addition, NEs can carry antigens to antigen presenting cells (APCs) much more efficiently than larger particles. Given their immune-stimulatory properties, BCG-CWCs in combination with NEs could facilitate antigen recognition by the innate immune system, an essential step for the development of a complete and robust adaptive immune response. Preparation of BCG-CWCs NEs required the isolation and sonication of BCG-CWCs prior to preparation of NEs by homogenization. We were able to determine that the average size of BCG-CWCs NEs is around 300 nm, with a surface potential of -50 mV. A concanavalin A binding assay showed that BCG-CWCs located on the surface of NEs. In an in-vitro monocytes-derived dendritic cells (DCs) assay, BCG-CWCs NEs increased the production of the pro-inflammatory cytokine, IL-12 and induced CD86 expression, a maturation marker of DCs. Immunofluorescent assays showed that BCG-CWCs were up-taken by APCs. The immunogenic properties of BCG-CWCs NEs point to a potentially effective adjuvant and antigen carrier. Further work is now required to measure NE stability and antigen loading efficacy.

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ESTABLISHMENT OF MOBILE LABORATORIES UP TO RISK GROUP 4 IN COMBINATION WITH CBRN CAPACITY BUILDING IN SUB-SAHARAN AFRICA

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Outbreaks with risk group 3 and 4 pathogens often occur in sub-Saharan Africa. Despite the considerable progress that African countries have made in outbreak management, there is still a need for international assistance in the detection, diagnosis and containment of infectious disease emergencies, such as viral hemorrhagic fevers (VHF). VHF often present with unspecific symptoms of febrile illness, thus making the rapid application of molecular diagnostic techniques for the detection of the pathogens concerned necessary. Therefore, the EuropeAid Cooperation Office of the European Commission has set up this collaborative project, with the overall aim to strengthen scientific cooperation between Europe and Africa in the field of epidemic-prone infectious diseases. Three rapidly deployable laboratory units as well as a collaborative network of European and African institutions will be established in the course of this project. A pool of African and European scientists will be trained in the use of these easy deployable and therefore highly mobile laboratory units, facilitating rapid field based diagnostic response to outbreaks of infectious diseases of risk group 3 and 4 pathogens. One mobile lab each will be stationed in Nigeria, Tanzania and Germany, respectively, and training missions, mock deployments and possibly outbreak response missions in the framework the Global Outbreak Alert and Response Network (GOARN) are planned. The project is linked with WHO, ECDC, and EU public health networks such as ENIVD and QUANDHIP. The European mobile lab consortium consists of partners from the Bernhard-Nocht-Institute, Bundeswehr Institute of Microbiology, Instituto Nazionale per le Malattie Infettive, Irrua-Specialist-Teaching-Hospital, National Institute for Medical Research Dar es Salam, Health Protection Agency, Institute of Virology Marburg, Laboratoire P4 INSERM, and Spiez Laboratory.

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CONSENT AND ASSENT IN PEDIATRIC RESEARCH IN TROPICAL MEDICINE

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International guidelines suggest that children provide assent for medical research in addition to their parent's consent. However, the concept of assent is confusing and lacks clarity. Assent is traditionally taken to mean "agreement" from someone who is not competent to provide consent. In pediatrics, this means that individuals below the age of majority (usually 18 years old) should provide assent if they are able to. We describe the current international debate surrounding the pediatric consent and assent process and the additional challenges arising when conducting pediatric

research in tropical medicine. These challenges are particularly complicated because diseases are acute, the burden of disease is high, there is a lack of resources and basic infrastructure, there are low levels of education and literacy, and the standard of healthcare is low. In addition, in such contexts, some children make adult-like decisions, are parents themselves or live in complex family situations. In this paper, we argue that the default position should be that competent children should be able to consent for themselves regardless of their age. A competent child who can make his or her own autonomous decisions should be allowed to do so - the required level of competence being relative to a specific decision. This implies, we argue, that the decision about whether to participate in a study, in cases where the decision is of comparable complexity to the decisions the child is used to making in their daily life, should be made by the child. This complexity should not be judged according to first world standards, but in relation to the local setting taking into consideration the benefits, risks and implications of the available choices the child can usually or sometimes make. As in high-income settings, incompetent children should assent in addition to parental consent - assent being involving the child to the extent compatible to his or her maturity and cultural norms, not getting the child's permission to proceed as advocated by current research guidelines. It is important that policies and decisions about particular studies, particular institutions or study sites should be justified, deliberations recorded and final decisions approved by relevant parties.

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DISEASE ERADICATION: IS AN ECONOMIC PERSPECTIVE USEFUL?

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The global health community pays renewed attention to evaluating the feasibility of elimination and eradication of communicable diseases. Given the intense competition for global health resources in deciding to commit to a national/regional elimination or eradication initiative, economic considerations are important. We developed a framework to show how economics can provide insights on disease eradication and we reviewed the evidence on economic literature. The framework is based on three key questions: 1) Why to eradicate? 2) How to achieve eradication? 3) For Whom? The "Why question" compares costs, health and economic benefits in the long run. The "How question", assesses which intervention/s or strategy/ies should be adopted by which stakeholder; how to generate incentives for each country to eliminate; how much resources would be required; and how these could be mobilized. The "for Whom question", assesses who would benefit from eradication, and the likely impact on equity and fairness. The impact of eradication is of long term. Thus, economic principles are key to assess how much value to give to future health and economic benefits and costs. Elimination and eradication are likely to benefit the most poor with consequences on equity and fairness. As it is not possible to exclude a country/community from the benefits of eradication and every country/community can benefit from it without limiting the others' benefits, disease eradication is a global public good. Economic theory can help assessing the feasibility of eradication by modelling the strategies stakeholders may take and the incentives required to cooperate. Most of the economic literature reviewed is, however, focused on comparing short term costs and consequences. The impact on economic development is rarely explored and issues of equity and fairness are neglected. Our review shows that while economic analyses can be powerful levers to support global eradication policies, methods adopted are based on reductionist approaches failing to consider relevant aspects.

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COMPARISON OF WRITTEN VERSUS ILLUSTRATED CONSENT METHODS IN A RESOURCE-LIMITED REGION OF THE PERUVIAN AMAZON

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Informed consent is vital to the ethical conduct of research involving humans. Proper informed consent includes a decision-making step by the research subject, following explanation of a study by research personnel knowledgeable in participants' rights. While multiple studies in the U.S. and Europe have compared various informed consent processes, this topic has received little attention in resource-restricted regions such as Peru. Our study aimed to compare comprehension, satisfaction, and length of time of the informed consent process using a text-only document versus an illustrated flip-book. Before enrollment in a febrile surveillance study in the Peruvian Amazon, 254 adults were randomized to receive one of the two consent methods. The two groups were comparable by age, gender, and level of education. Both methods contained identical information and all the study personnel were trained to administer informed consent in a standardized fashion. After subjects finished the consent process, a 17-item questionnaire was administered that measured recall of particular facts about the study and satisfaction with the consent process. We found that those consented with the illustrated method did not have a different level of comprehension than those consented with the written method when we compared the average number of correct answers per subject or the proportion of correct answers for each individual question. Both methods also had similar satisfaction rates and length of consent time (25 minutes for written, 27 minutes for illustrated). Although our results indicate that the two consent processes performed similarly, an illustrated consent form may still offer unmeasured advantages, such as a consistent consent presentation in multi-centric studies and easier comprehension for illiterate study subjects.

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INCREASING SUPPLY CHAIN KNOWLEDGE, SKILLS AND AVAILABILITY OF TOOLS AMONG HEALTH EXTENSION WORKERS (HEWS) TO IMPROVE COMMUNITY HEALTH SUPPLY CHAIN IN ETHIOPIA

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In Ethiopia, integrated community case management is provided by health extension workers (HEWs) who are trained to provide 16 packages of preventive and curative health services. A baseline assessment identified a lack of basic supply chain management knowledge and skills among HEWs. The SC4CCM Project designed a group training approach, comprising of Integrated Pharmaceutical Logistics System (IPLS) Ready Lessons and Problem Solving (PS) modules, which used existing resources and time by incorporating trainings into routine health center meetings. The intervention aimed to provide affordable, maximum coverage of supply chain management knowledge, skills and tools among HEWs to ensure basic processes and competencies. Training effectiveness was measured using a competency questionnaire, where HEWs were presented with a mock situation and completed a test that assessed components of the IPLS and PS modules. After six months of implementation, the intervention group was characterized by a more rapid pace of IPLS training

rollout to HEWs and greater availability of key supply chain tools including a training manual and blank reporting tools compared to the comparison group. Competency assessments yielded minimal differences across training methods, with HEWs in both groups having difficulty executing complex recording and reporting tasks. Qualitative data from both groups showed that training was not conducted uniformly across regions and that HEWs stated the need for repeated training on more complex topics. Results also showed that specific components of training were found to be effective including problem solving, practical trainings including demonstration, refresher trainings, supportive supervision and regular review meetings. Additionally, minimal differences in competencies could be due to the evaluation being conducted only six months post-training. We therefore concluded that repeated competency testing over time and triangulation of data using other methods such as observation will help supplement and better assess training effectiveness.

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PAPER-BASED WHOLE CELL BIOSENSORS FOR INEXPENSIVE AND SPECIFIC PHARMACEUTICAL QUALITY TESTING

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Substandard pharmaceuticals have been estimated by the World Health Organization to be 30% of the pharmaceutical market in developing countries. This prevalence of poor quality drugs persists despite an abundance of state of the art analytical methods that exist today. To best address this problem in low resource settings, inexpensive and field-friendly technology is required. Paper-based tests have long been a user-friendly solution to many analytical problems. We have incorporated genetically engineered, whole-cell yeast biosensors into paper, using hydrogels, to make highly specific tests for antibiotics in the tetracycline family. This strain of Saccharomyces cerevisiae are engineered to repress reporter expression in the absence of tetracycline drugs and up-regulate expression in their presence, producing a tightly controlled indicator for this family of antibiotics. Incorporating these yeast into a paper device produces a low-cost, easily transportable test that would have the specificity of a biological system, an improvement over current chemical tests which recognize functional groups, without the need for isolation of antibodies or cell components. Stability testing reveals that these tests remain responsive to analyte for 6 month with refrigeration and can withstand at least 5 days at 47°C. We find that this technology is able to identify the presence of doxycycline at concentrations of 100-3,000 µg/mL. The low-cost nature and portability of this analytical device make it a viable option for use in detecting antibiotics of the tetracycline family in pharmaceuticals in developing countries where the quality of medicines is of concern.

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POTENTIAL OF NUCLEAR QUADRUPOLE RESONANCE SPECTROSCOPY FOR DETECTION AND CHARACTERIZATION OF COUNTERFEIT MEDICINES

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Counterfeit medicines pose a global and vast growing threat for patients. According to the World Health Organization (WHO), the definition of counterfeit medicines highlights a deliberate and fraudulent mislabelling of products. The development of methods for the identification of medicines is extremely important. Nuclear Quadrupole Resonance (NQR) spectroscopy characterizes solid-state substances containing quadrupolar nuclei (spin quantum number $I > ½$). Since approximately 90% of medicines are in solid form, NQR can detect and identify signals from a broad range of medicines (^{14}N, ^{35}Cl, ^{23}Na etc), focusing only on the active pharmaceutical ingredient (API). This abstract reports the study of detection and characterization of different nitrogenous medicines in the form of tablets, capsules and powder. The aim is to test the NQR response of medicines of different formulations. A quantitative study of classification of a real and suspected counterfeit anti-malarial medicine Metakelfin has also been contacted. A sequence of radio-frequency pulses leads to sample excitation, following the acquisition of the emitted signal. In this study, the "Pulse-spin locking" (PSL) multiple-pulse sequence is used. The linewidth of the NQR signal is described by its characteristic relaxation time constant T_2^* that infers information about the mechanical processing of the medicine. Analgesic Paracetamol (acetaminophen) caplets, capsules and powder of the same brand and quantity, were detected at 2.5637MHz. Variation in the linewidth suggests that NQR infers information about the crystal structure of the sample, specifying the formulation and manufacturing processing of the medicines. The ^{14}N sulfalene component of suspected metakelfin (3.075MHz) tablets is compared with the signal from the genuine batch. The quantitative analysis of the suspected product shows that the sulfanene content is 2.1 times lower than the genuine metakelfin tablets. This study indicates the ability of NQR to detect quantitatively suspected drugs, and the potential of generating drug fingerprints.

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IMPROVING DATA TO IMPROVE PROGRAMS: IMPLEMENTATION OF A DATA USE PACKAGE AS PART OF THE COMMUNITY CASE MANAGEMENT PROGRAM FOR COMMON CHILDHOOD ILLNESSES IN MALAWI

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The "Implementation Research Embedded in Integrated Community Case Management (CCM) Program: Improving Data to Improve Programs (CCM-IDIP)" Translating Research into Action (TRAction) project is working with CCM programs in Malawi and other countries to improve monitoring, evaluation and use of information. Through a desk review and data quality assessment of the current CCM M&E system, we found a well-defined structure for routine reporting and good levels of reporting and completeness. However data use is low and mostly a top-down approach. Community health workers and their supervisors expressed a keen interest in understanding and using the data that they are collecting and reporting. We worked with district health staff and partners to develop a program to increase data interpretation and use at the community, health center and district levels. By increasing data use at lower levels of the health system, we are hoping to not only improve the overall M&E data quality but allow health workers to quickly make data-based decisions to improve programs. The aim of the package is to improve data use and quality by giving community health workers, health facility and district staff the tools to analyze and interpret the M&E CCM data they routinely report. The package includes (1) general training on data management, use and interpretation; (2) refresher training on the routine reporting forms; (3) simple templates for displaying the monthly CCM implementation strength data; (4) provision of calculators to assist with completing monitoring forms; and (5) working with district staff to identify reporting benchmarks and action thresholds. Since February 2013, two districts in Malawi have been implementing a data use and improvement package. District health staff were trained to provide training on the data use package to the health workers. All CCM-trained workers in the two districts were targeted for training on data use and the package will be implemented for at least three months. A supervisory field mission at the midpoint of implementation show that the templates are being used at all levels and

health worker feedback is on the package is very positive. An endline RDA will be conducted in May 2013 to evaluate whether the program improves data use and quality.

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A FOURTH DELAY: MALARIA PREVENTION AND NUTRITIONAL SUPPLEMENTATION IN THE FIRST TRIMESTER FOR PREGNANT WOMEN IN BURKINA FASO

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In Burkina Faso, the infant mortality rate is 91.7 per 1,000 live births and the maternal mortality rate is 307.2 per 100,000 live births. Hemorrhage is the direct cause of more than 30% maternal deaths in Burkina Faso each year. Malaria, anemia, HIV/AIDS, and hemoglobinopathies are indirectly responsible for 20% of maternal deaths in Burkina Faso. Literature cites three delays contributing to these maternal deaths namely delays in (1) the decision to seek care, (2) arrival at a health facility and (3) receiving appropriate care upon arrival. This study assessed sociocultural barriers to preventative care for Burkinabé women. From October 2012 to May 2013, focus-group discussions and semi-structured interviews were conducted with pregnant or postpartum women, aged 17 to 40, in one rural and one urban maternity clinic in the medical district of Bogodogo in Burkina Faso. All discussions were recorded, transcribed, analyzed in QSR N-Vivo 10 and then translated into English for the sake of reporting results. Of 60 Women interviewed, 90% cited prenatal visits among the most important steps a pregnant woman should take. However, few women reported attending more than two of the recommended four to six prenatal visits for their current or previous pregnancies and a majority of women believed it was not recommended to have a prenatal visit before the end of the first trimester. Many women claimed that early announcement of pregnancy and prenatal visits would be viewed negatively by other community members and could compromise their health. This delay in prenatal visits translates to a delay in iron and folic-acid supplementation in a population already at risk for anemia in pregnancy and hemorrhage during delivery. None of the women who reported owning insecticide-treated nets claimed to use them every night. The primary reasons women cited for not using mosquito nets were perceived suffocation and oppressive heat. This study suggestions that public awareness campaigns should encourage women to seek early prenatal care to ensure early nutritional support, malaria prophylaxis and counseling in the use of insecticide-treated mosquito nets.

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MEASURING THE STRENGTH OF COMMUNITY CASE MANAGEMENT IMPLEMENTATION: VALIDATION OF MOBILE PHONE INTERVIEWS WITH COMMUNITY HEALTH WORKERS IN MALAWI

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Since 2008, Health Surveillance Assistants (HSAs) have provided community case management (CCM) of childhood malaria, diarrhea and pneumonia episodes in selected areas of Malawi. In order to gauge the level of program implementation and provide continuous feedback for program improvement, implementers and evaluators require high-quality and inexpensive measures of implementation strength. However the routine CCM monitoring and evaluation system is still being scaled-up in many areas. We have tested and validated a method for collecting implementation strength data at the community level through mobile phone interviews with HSAs. We conducted telephone interviews with 241 CCM-trained HSAs in two districts of Malawi covering training, supervision, utilization and drug stocks. The HSA responses were then validated through direct observation of records at the health centers, drug stocks and CCM registers at the village clinics. A short qualitative module was administered to the HSA at the end of the interview/observation to determine the reasons for any observed discrepancies. We calculated the sensitivity and specificity for key implementation strength indicators based on the cellphone interview using record review/observations as the gold standard. A large proportion (83%) of HSAs were available for mobile phone interview despite recurring network issues. We found sensitivity and specificity for the cell phone interview method to be very high for CCM training status, receipt of initial drug box and provision of services (above 95% for all). Supervision and mentoring indicators were a bit lower although still acceptable (above 80% for all). Current drug stocks and minimum level of key CCM drugs were also high (above 90%). The sensitivity/specificity of the interview method for reported drug stock-outs in the previous three months were a bit lower especially for anti-malarials. We found similar levels of sensitivity and specificity across districts. Many of observed discrepancies were due to HSA errors during the interview such as counting errors, poor understanding of the questions due to network interference and recall mistakes. This study showed that mobile phone interview directly with HSAs provides accurate information on implementation strength, and represents a feasible, low-cost approach of measuring implementation strength indicators in areas where the routine M&E system is still being scaled-up.

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ENGAGING THE PUBLIC IN PUBLIC HEALTH: THE OPPORTUNITIES AND CHALLENGES OF PARTICIPATORY SURVEILLANCE

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Over the past decade, internet-based participatory surveillance systems for influenza have proven to be fast, accurate, and sensitive. They are also highly scalable, offering the possibility of moving into new areas and targeting more diverse diseases. SaludBoricua.org, a new participatory surveillance system in Puerto Rico, was designed to target multiple acute febrile illnesses - influenza, dengue, and leptospirosis - in an environment where all are present. We are evaluating the ability of self-reported symptoms to differentiate these diseases by comparing participant-generated data to data from traditional, healthcare-based systems and by evaluating data collected in acute febrile illness studies. The system also offers the opportunity to evaluate the impact of interventions, such as vaccination or vector-control, on disease outcomes and characteristics of healthcare system utilization. Moreover, participatory surveillance offers the opportunity to engage the public directly with public health. The public contributes to and has access to the aggregate data and the system forms a direct communication link between public health authorities and the public. Engagement of the public is also one of the largest challenges as these systems must attract users, encourage continued participation, provide useful information, and establish credibility.

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THE IMPLEMENTATION OF OHASIS, A COMPREHENSIVE HEALTH INFORMATION SYSTEM IN A SOUTH AFRICAN HEALTH LABORATORY SERVICE

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OHASIS is an occupational health information system which gathers data on worker health assessments, hazards and incidents in the workplace. It assists decision makers, health and safety committees and researchers to monitor employee health and safety. OHASIS was developed by the Global Health Research Program (GHRP) at the University of British Columbia (UBC), Canada and installed at the National Health Laboratory Service (NHLS) who embarked on a process of capacity building and empowerment to develop local expertise in the ongoing development thereof. NHLS employs about 7000 employees in 349 pathology laboratories across South Africa and 2 National Institutes. Laboratories range from BSL2 to BSL4. The relationship and co-operation between UBC and the NHLS has been used to implement and strengthen OHASIS in other sites globally. OHASIS has various modules, incident reporting and investigation, workplace assessment and employee health with special reference to HIV and TB. There are complexities in implementation in a resource limited setting and the progress of the system from a paper based to an online system together with the training methods used will be discussed. OHASIS was applied in September 2011 and 413 incidents were reported to date. This is approximately 270 per year, giving an incident rate of 39/1000, an increase compared to the previous 2 years with rates of 33 and 35/1000. Taking incident reporting online should improve reporting further. A survey of health and safety services offered to employees prior to the implementation of OHASIS online has been conducted with 316 respondents showing about 17% required more training with a focus on HIV and TB, 3% indicated that they would never report an incident and 20% conflicted. An e-learning platform is being used to train employees on the online use of OHASIS. Information will be shared on modules being developed like the tracking of hazardous medical waste, facility auditing and equipment maintenance and the interlinking of modules. Introduction of OHASIS in Ghana is being considered.

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SAVING RAINFOREST WITH A STETHESCOPE: FIVE-YEAR ASSESSMENT OF THE IMPACT OF HEALTH IN HARMONY'S HUMAN AND ENVIRONMENTAL HEALTH INTERVENTIONS IN BORNEO, INDONESIA

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Project ASRI integrates high quality, affordable health care with conservation strategies in Gunung Palung National Park (GPNP) in Borneo, Indonesia. We seek to determine whether the 35,000 patient visits to Klinik ASRI, 1500 mobile clinic visits, ambulance service, community health workers for DOTS TB management, and various livelihood training programs have affected human and environmental health of the region. We conducted a baseline (2007) and follow-up (2012) demographic and KABP survey of 25 villages surrounding the GPNP. Pairs of trained nurses systematically interviewed 1300 households out of 60,000 in the region. Overall health of the population improved. Under 5 mortality was reduced 14.9%; rates of child immunizations increased 25.4%; symptoms of diarrhea, fever, cough < 3 weeks and weight loss were reduced (49.4, 26.2, 59.7, and 68.4% respectively); mosquito net use increased 11.7%; number of births per mother decreased 16.1%; use of birth control increased 12.0%; and, access to water pipes, a restroom and boiling water prior to drinking increased (15.0%, 21.5% and 24.9% respectively). 30 of 32 villages were engaged in ASRI's organic farming, animal husbandry and reforestation programs for livelihood alternatives to illegal logging and there was a 68% decrease in the number of people who illegally logged. ASRI has improved the overall health of the region: ASRI patients were more likely to be healthy and illegal logging is declining.

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PEACE CORPS VOLUNTEERS SUPPORT INTERACTIVE YOUTH AND COMMUNITY ACTIVITIES FOR WORLD MALARIA DAY - KENYA, 2013

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In 2011, Peace Corps and the President's Malaria Initiative launched Stomping Out Malaria in Africa. In 2012, three Peace Corps Volunteers (PCVs) serving in Kenya received 4 weeks of specialized malaria training to become Malaria Volunteers (MVs). The MVs work with communities, health facilities and the Government of Kenya (GoK) to implement malaria prevention and treatment activities focusing on at-risk and vulnerable populations such as children aged 5-14 years, who have the highest prevalence of malaria in Kenya. The MVs recruited PCVs to implement local World Malaria Day (WMD) activities to increase youth and community awareness of malaria. Participating PCVs attended malaria educational sessions and developed WMD community event plans. Each PCV received a promotional item pack, which included t-shirts, bags and footballs with GoK malaria messaging. Packs were procured and distributed by a partner organization. Limited numbers of socially-marketed insecticide-treated bed nets were provided for resale at community events. Inclusion of community-based organizations, vulnerable populations and GoK staff in the planning process was encouraged. Seventy (61%) of 115 PCVs serving in Kenya planned events in 45 communities across 32 districts. Fifty-eight (87%) PCVs worked in education or health sectors. Sixty-seven (96%) events included school and community football tournaments as part of activities. Fifty-seven schools, 41 community-based organizations, including 10 orphans and vulnerable children's groups, were expected to participate. The participation target was over 8,000 youth, teachers and community members for an average of 120 people per PCV. Football tournaments were held to increase primary and secondary school youth participation in WMD activities. Increasing youth and community awareness of malaria and other health issues by leveraging community-based PCVs to host interactive activities is a strategy that should be more widely adopted.

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SOLUBLE *PLASMODIUM* APOPTOTIC FACTORS INDUCE APOPTOSIS IN BRAIN VASCULAR AND HEMATOPOIETIC STEM CELLS

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The severity of malaria caused by *Plasmodium falciparum* is determined by many elements, including both parasite apoptotic and host inflammatory factors. One such factor, *P. falciparum* apoptotic factor-1 (PfAF-1), is a soluble factor of parasitized erythrocytes (pRBC) that induces apoptosis in human brain microvascular endothelial cells (HBVEC) and neuroglia. Other factors include submicron vesicles (SV) shed from the plasma membrane of eukaryotic cells and free heme (Hemin) produced during malaria infection. These factors have various effects on vascular endothelia, the blood brain barrier (BBB) and circulating endothelial progenitor cells (cEPC). Serious complications of *P. falciparum* malaria infection are brain endothelial cell damage resulting in BBB dysfunction as well as decreases in endothelial precursors, cEPC, responsible for vascular repair. The independent roles

of these factors in malaria severity are unclear. In addition, Hematopoietic Stem cells (HSC), representative precursors of cEPC, may be susceptible to the apoptotic effects of aforementioned soluble factors, but have not been determined. Our hypothesis of this study was that *P. falciparum* induced apoptotic factors alter cell viability and apoptosis in HBVEC and HSC *in vitro*. HBVEC and HSC viability was assessed using MTT assay and apoptotic indices were measured by activated caspase-3 expression and DNA fragmentation. Significant decreases ($p<0.05$) in viability and increases ($p<0.05$) in apoptosis were observed in PfAF-1, Hemin and SV treated HBVEC and HSC versus non-treated controls. *P. falciparum* apoptotic factors play an important role in inducing apoptosis in HBVEC and HSC. The depletion of cEPC contributes to the serious complication of malaria. *P. falciparum* apoptotic factors could be novel therapeutic targets for severe malaria through decreasing BBB damage and preventing cEPC depletion.

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POLYMERASE CHAIN REACTION AND HISTOLOGY IN DIAGNOSIS OF PLACENTAL MALARIA IN AN AREA OF UNSTABLE MALARIA TRANSMISSION IN CENTRAL SUDAN

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Prevalence of placental malaria has been widely used as a standard indicator to characterize malaria infection in epidemiologic surveys. Placental malaria poses a greater diagnostic challenge, accurate and sensitive diagnostic tool for malaria infections in pregnancy is needed. A cross sectional study was conducted at Medani Hospital, which serves catchment area which is characterized by unstable malaria transmission. One hundred and seven placentae were investigated for malaria infection using polymerase chain reaction (PCR) and histology. Out of 107 investigated placentae, 33 (30.8%) and 34 (31.8%) were positive for malaria by histology (two (2%) and 31(29.0%) were acute and past infections, respectively) and PCR, respectively. Out of 33 positive by histology, 15 were positive by the PCR while 18 were negative. The sensitivity of the PCR was 45.5% (95% CI: 29.2%- 62.5%). Out of 74 which were negative by histology, 19 were positive by the PCR. This is translated in specificity of 74.3% (95% CI: 63.5%- 83.3%). Of those tested positive by the PCR, 15 were positive by the histology, while 19 were negative. This is translated into a positive predictive value of 44.1% (95% CI: 28.3%- 61.0%). Of those 73 tested negative by the PCR, 55 were negative according to histology while 23 were positive. This is translated into a negative predictive value of 75.3% (95% CI: 64.5%-84.2%). In conclusion, PCR had low sensitivity and specificity in comparison to placental histology, perhaps because the vast majority of the placental infections were past infections. Further research is needed.

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GENOTYPING OF *PLASMODIUM FALCIPARUM* USING AN AGILENT 2100 BIOANALYZER

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The simultaneous infection of hosts by multiple parasite clones of the highly genetic diverse *Plasmodium falciparum* parasite complicates the understanding of malaria infection. PCR based genotyping of the genes encoding the merozoite surface proteins 1 and 2 (*msp1* and *msp2*) and the glutamate-rich protein (*glurp*) which show high level of polymorphism has been well established to determine the number of parasite clones within one host. Fragment analysis is performed using either gel electrophoresis or capillary electrophoresis (CE). CE has been shown to be highly discriminative compared to gel electrophoresis. This method is however expensive and time consuming. Here we explore the feasibility of using the Agilent 2100 Bioanalyzer, a microfluidic chip-based platform in genotyping *P. falciparum*, and compare the accuracy of the results to capillary electrophoresis in counting the number of clones per infection.

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INCREASED CEREBROSPINAL FLUID SUPEROXIDE DISMUTASE ACTIVITY IS ASSOCIATED WITH INCREASED SEIZURE ACTIVITY AND PROLONGED DURATION OF COMA IN CEREBRAL MALARIA

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To elucidate the role of oxidative stress in severe malaria pathogenesis and outcomes, plasma Cu/Zn superoxide dismutase (SOD) concentration and activity were assessed in Ugandan children with cerebral malaria (CM, n=172), severe malarial anemia (SMA, n=142), and community children (CC, n=127). In children with CM, plasma and cerebrospinal fluid (CSF) SOD concentration and activity were then compared to mortality and central nervous system (CNS) outcomes. Oxidative stress leads to increased SOD concentration, but SOD activity may reflect ongoing oxidative stress or may be quenched with high levels of ROS. Plasma SOD concentration (median level, ng/ml, [25th, 75th percentile]) was higher in children with CM (322.1, [209.4, 489.4]) than children with SMA (210.6, [138, 320]) (P<0.0001) or CC (194, [138.8-311.4]) (P<0.0001), while levels in CC and SMA did not differ significantly (P=0.6). Plasma SOD activity (mU/ul) was decreased in CM (291, [91.9, 693.5]) and SMA (353, [168, 610.8]) compared to CC (645, [319, 1252.8]) (P<0.0001), but did not differ between CM and SMA (P=0.6). Children with CM who died had an elevated plasma SOD concentration and reduced plasma SOD activity (P=0.05 for both). Among children with CM, those who had seizures after admission had higher CSF SOD concentration (P=0.04) and activity (P=0.006), and CSF SOD activity also correlated positively with number of seizures after admission (Spearman's rho=0.23, P=0.005) and duration of coma (rho=0. 27, P=0.001). CSF SOD activity was inversely correlated with plasma SOD activity (rho=-0.26, P=0.003). Plasma or CSF SOD concentration or activity did not correlate with neurologic deficits at discharge or 6-month follow-up. Plasma SOD concentration is increased and activity decreased in children who die of CM as compared to survivors, but increased CSF SOD activity correlates with increased seizure activity and prolonged coma duration. CNS oxidative stress, as indicated by increased CSF SOD activity, may lead to increased CNS complications in children with CM.

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SEVERE MALARIA AND MILD MALARIA ARE ASSOCIATED WITH DISTINCT SUBSETS OF *PLASMODIUM FALCIPARUM*

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Malaria can manifest as either severe malaria or mild malaria in children, but the basis for different outcomes is not known. Our epidemiologic studies indicate that high parasite density alone cannot explain severe disease, and we speculated that "severe malaria parasites" have distinct features that increase their virulence over "mild malaria parasites". In microarray and RNA seq analyses, severe malaria parasites from Tanzanian children have distinct signatures. Some of the top differentially regulated genes have undefined roles in the parasite and may be involved in virulence related functions. Gene enrichment analyses coupled with detailed in silico investigation of parasite metabolic pathways indicate that some pathways may be altered in severe malaria parasites, including

enzymes of the purine metabolic pathway and the fatty acid biosynthetic pathway. These pathways may be involved in some aspects of severe disease and their roles are currently under investigation. Unexpectedly, severe malaria parasites also overexpress the variant surface antigen known as VAR2CSA, which was previously shown to be preferentially expressed by pregnancy malaria parasites and involved in adhesion to the placental receptor CSA. Our data suggest that VAR2CSA may have additional roles in disease pathogenesis for pregnant women and children. Differential gene expression in severe and mild malaria parasites has been further confirmed by qPCR analysis in samples isolated from a geographically distant site in Mali. Overall our study indicates that severe and mild malaria parasites display consistent gene expression differences that may be exploited towards therapeutic uses.

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ANGIOTENSIN II RECEPTORS INHIBIT *PLASMODIUM FALCIPARUM*-INDUCED DISRUPTION OF ENDOTHELIAL CELL JUNCTIONS AND PROTECT MICE AGAINST CEREBRAL MALARIA

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Cerebral malaria (CM) is a complication of severe malaria that leads to the disruption of the blood brain barrier and frequently results in death. We have found that angiotensin II receptors modulate the brain endothelial cell response to *Plasmodium falciparum*-infected erythrocytes by preserving the integrity of interendothelial cell junctions and protecting against experimental CM. We have seen that the disruption of junctions caused by rupture of infected erythrocytes over human brain endothelial cells *in vitro* is inhibited in the presence of blockers of angiotensin II receptor 1 (AT1) or activators of angiotensin II receptor 2 (AT2). Complementary results in the CM mouse model show that treatment of mice with blockers of AT1 or activators of AT2 does not affect levels of *parasitemia*, but result in highly increased survival. Conversely, transgenic mice lacking AT2 receptor present increased susceptibility to CM. These results may facilitate clinical applications, especially since Losartan, an AT1 blocker that showed protective activity *in vitro* and *in vivo*, is commonly used as treatment for hypertension in humans.

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IMPAIRED RED CELL DEFORMABILITY IN *KNOWLESI* MALARIA IN PROPORTION TO DISEASE SEVERITY

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Plasmodium knowlesi commonly causes severe and fatal malaria in Malaysian Borneo, but little is known about the pathogenesis of disease. In severe *falciparum* malaria, sequestration of parasitized red cells results from cytoadherence to host endothelium and decreased red blood cell deformability (RBC-D), leading to microvascular obstruction, tissue hypoxia and organ dysfunction. In *knowlesi* malaria microvascular accumulation of parasitized cells also occurs, however the mechanisms are unknown. Reduced deformability of *P. knowlesi*-infected red cells has been demonstrated in rhesus macaques, but has not been studied in humans. Using ektacytometry we measured RBC-D in adults with severe (n=21) and non-severe (n=61) *knowlesi* malaria and severe (n=8) and non-severe (n=82) *falciparum* malaria; and 15 healthy controls. At a shear stress of 30 Pascals, RBC-D was reduced in patients with severe (elongation index [EI]=0.496, IQR 0.456-0.528) and non-severe (EI=0.551, IQR 0.494-0.569) *knowlesi* malaria compared to controls (EI=0.583, IQR 0.576-0.590; $p<0.0001$ for both comparisons), and reduced in severe compared to non-severe *knowlesi* malaria ($p=0.002$). RBC-D was similar among patients with severe (EI=0.510, IQR 0.496-0.539) and non-severe *falciparum* malaria (EI=0.516, IQR 0.475-0.558), but was reduced in both groups compared to controls ($p=0.0045$ and $p<0.0001$ respectively). RBC-D did not differ significantly between patients with severe *knowlesi* and severe *falciparum* malaria. Among patients with *knowlesi*, but not *falciparum* malaria, RBC-D was inversely correlated with parasite count (spearman's correlation coefficient =-0.37, $p=0.0006$). Among patients with *knowlesi* malaria, reduced RBC-D may contribute to microvascular sludging, microvascular accumulation of parasitized red cells and impaired organ perfusion in severe disease.

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A NEW APPROACH TO THE MANAGEMENT OF SEVERE ANEMIA IN *PLASMODIUM FALCIPARUM* INFECTION

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Rupture of invaded red blood cells as they release merozoites into the blood circulation, is a cause of the anaemia in *Plasmodium falciparum* infection. There appears to be yet another and perhaps more serious mechanism that contributes to the severe anaemia seen in *P. falciparum* infection. Some patients on blood transfusion (whole blood or packed cells) for severe anaemia in *P. falciparum* infection have been observed to return to square one (became pale again) within 24 to 72 hours of such transfusion. Giving more blood never changed the situation as they always returned to square one. The issue of jaundice seen in some of these cases tends to start when the spleen began to enlarge and did not correspond to the degree of anaemia as it was usually mild. In some of these patients, there was no jaundice, the level of bilirubin in the blood was normal and there was no urobilirubin in the urine. Perhaps the severe anaemia in *P. falciparum* infection is due to a phenomenon of massive pooling of un-invaded red blood cells from the peripheral circulation into some capillary beds the liver and/or the intestine. This may be an auto-protective mechanism to prevent these cells from being invaded by the *P. falciparum* merocites as they are released into the circulation from the liver. The anaemia in all these cases of severe anaemia that returned to square one after blood transfusion was corrected by adequately treating the malaria and reversing the Auto-Protective massive pooling of un-invaded red blood cells from the peripheral circulation, without further blood transfusion. Perhaps the solution to the management of severe anaemia in *P. falciparum* Infection is not Blood Transfusion but adequate treatment of the malaria fever and the reversal of the auto-protective massive pooling of un-invaded red blood cells from the peripheral circulation phenomenon. The need to investigate the presence of a possible auto-protective massive pooling of un-invaded red blood cells from the peripheral circulation, accounting for the severe anaemia in *P. falciparum* infection can therefore not be over emphasized.

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COINFECTION OF MURINE GAMMA HERPES VIRUS AND *PLASMODIUM YOELII*: IMPACT ON HOST RESPONSE AND DISEASE SEVERITY

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EBV, a gamma herpes virus and *Plasmodium falciparum* co-infections in early childhood have been associated with the development of endemic Burkitt's lymphoma (eBL), the most common form of pediatric cancer in

equatorial Africa, accounting for nearly 70% of childhood malignancies in these areas. The mutual interaction between viruses and malarial parasites are poorly understood. In order to understand the interaction between gamma herpesvirus and malaria, we established a co-infection model that involves infection of mice with murine gamma -herpesvirus (MHV-68) and *P. yoelii* non-lethal strain (PY17XNL), a murine malaria parasite. Co- infection of MHV68 infected mice with *P. yoelii* results in uncontrolled *parasitemia* and severe anemia (low Hb levels) versus mice singly infected with *P. yoelii*, which readily clears the infection (*parasitemia*) post 3 weeks of infection. Pronounced splenomegaly was observed in co-infected mice accompanied with severe weight loss as compared to singly infected mice. Major alterations in host immune responses and significant perturbations in B and T cell populations were observed. Significantly lower levels of B cells, plasma cells, germinal center cells, (CD4+ and CD8+) T cells were seen in co-infected mice as compared to *P. yoelii* singly infected mice. Our data has demonstrated strong synergy between dual/co infected mice with MHV68 and *P. yoelii*, providing an experimental model in which interference in normal host control of both gamma herpes virus and plasmodium infections are observed. The insights gained from this study may help in understanding the alterations in host responses and gamma herpes virus pathogenesis in co-infected individuals that predispose children to develop Burkitt's lymphoma.

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PATTERNS OF LEAKAGE FROM RETINAL VESSELS IN PEDIATRIC CEREBRAL MALARIA

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In pediatric cerebral malaria (CM) death and neuro-disability are associated with both retinal vessel leakage and cerebral edema on MRI. We used fluorescein angiography (FA) to investigate presence and severity of leakage from the retinal neurovasculature. We performed FA in the pediatric research ward in Blantyre, Malawi, on children with a clinical definition of CM admitted between 2006-2010. The presence of any leak on admission images was determined by primary grading. Secondary grading assessed detailed leakage subtype and severity on a consecutive set within the series. We report data from the left eye. Leakage was present in 125/170 subjects. In secondary grading (n=87) three main leakage subtypes were found in three mutually exclusive retinal areas (area, % of cases): vessel segment leak (macula 87%, raphe 85%, periphery 89%), focal leak (macula 3%, periphery 15%), punctate multifocal leak (macula 3%, periphery 13%). Each subtype varied in severity, could occur in multiple regions, and could coexist with other subtypes. Vessel segment leak was almost exclusively seen in post-capillary venules and capillaries - only one case had leakage from arterioles. Focal leakage involved areas of 100-500µm in greatest linear diameter and occasionally occurred in large numbers, up to 15. Serial images during admission suggest this is an initial phase of hemorrhage development. Punctate multifocal leak appeared to involve significant dye leakage through very small segments of capillaries, and was often widespread. Unlike focal leak, punctate multifocal leak did not appear to precede hemorrhage. Pediatric CM frequently involves retinal neurovascular leakage, with a wide range of severity. The existence of leakage subtypes suggests that CM may affect the blood-retinal barrier in multiple ways. Insofar as the retinal and brain neurovasculature are similar these subtypes could also reflect brain pathogenesis.

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ENDOTHELIN-1 TREATMENT INDUCES EXPERIMENTAL CEREBRAL MALARIA DURING *PLASMODIUM BERGHEI* NK65 INFECTION

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Human cerebral malaria (CM) is a life-threatening complication of *Plasmodium falciparum*. Infection of C57BL/6 (B6) mice with *P. berghei* ANKA recapitulates many aspects of CM and is a widely used experimental cerebral malaria (ECM) model. Infection of B6 mice with *P. berghei* NK65 (PbN) does not induce neurological complications. Endothelin-1 (ET-1) is a potent vasoconstrictor with chemotactic properties for leukocytes involved in the pathogenesis of neuroinflammatory diseases. Blockade of the endothelin receptor A prevents the development of ECM suggesting that ET-1 contributes to its pathogenesis. We hypothesized that exogenous treatment of PbN-infected mice with ET-1 triggers the development of ECM. Mice were infected with 106 PbN-parasitized red blood cells and treated with either ET-1 or saline from 4 to 8 days post infection (dpi). PbN-infected mice treated with saline (n=20) did not display ECM and survived until 12 dpi, whereas PbN-infected mice treated with ET-1 (n=18) exhibited signs of ECM such as ataxia and died 4 to 8 dpi. ET-1 treatment had no effect on *parasitemia*, except at 5 dpi when ET-1-treated mice displayed a significant increase in *parasitemia* compared with saline-treated mice. Infected mice had a significant reduction in rectal temperature (RT) and body weight (BW) over the course of infection and the reduction in these parameters was significantly greater in PbN-infected mice treated with ET-1 (>20% weight loss). Uninfected mice treated with ET-1 had a smaller reduction in RT but not in BW. Brain histopathology of PbN-infected mice treated with ET-1 demonstrated the presence of petechial hemorrhages throughout the parenchyma and leukocyte infiltration to the endothelia 6 dpi which were not evident in PbN-infected mice treated with saline or uninfected mice treated with ET-1. These data indicate that ET-1 triggers the development of ECM in PbN-infected mice.

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POLYAMINE BIOSYNTHESIS ENZYMES ARE CRITICAL FOR THE DEVELOPMENT OF THE MALARIA PARASITE IN THE MAMMALIAN AND MOSQUITO HOSTS

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Polyamines are important organic charged molecules that play important roles in the cell cycle regulation, cell proliferation, senescence and death of eukaryotes and prokaryotes. In addition, polyamine analogues have been considered and applied in cancer therapy. Despite of the constitutive expression of polyamine biosynthesis enzymes during all malaria parasite life cycle stages, little is known about their biosynthesis and cellular functions for *Plasmodium* development in the mosquito and the mammalian hosts. Herein, we applied gene-targeting techniques in *P. yoelii* to target enzymes of this pathway for deletion and for fluorescent tagging, with or without the supplementation of polyamines. Our results indicate that polyamines biosynthesis is critical for the development of life cycle stages of *Plasmodium* in the mammalian and the mosquito hosts. Therefore, our data suggest the potential of polyamine biosynthesis enzymes as multistage drug targets for antimalarial chemotherapy.

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A POISSON HIERARCHICAL MODELLING APPROACH TO DETECTING COPY NUMBER VARIATION IN THE *PLASMODIUM FALCIPARUM* GENOME USING SEQUENCE COVERAGE DATA

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Next generation sequencing technology has made possible to identify copy number variation (CNV) in a large number of *Plasmodium falciparum* genomes. However, current CNV detection methods rely on statistical assumptions that do not hold in available *P. falciparum* sequencing data, or require fine-tuning the underlying algorithms, a task that may not be feasible when there are a large number of samples under analysis. We propose a new CNV detection methodology based on two Poisson hierarchical models, the Poisson-Gamma and Poisson-Lognormal, with the advantage of being sufficiently flexible to capture different data patterns and with stringency controlled by a statistical parameter similar to the significance level used in traditional statistical analysis. Using 3D7 resequencing coverage data and simulation, our methodology showed a baseline false positive rate in line with the stringency adopted for the analysis. When applied to the non-reference isolate data (HB3, DD2, 7G8, GB4, OX005, OX006), our approach detected known CNV hits, including an amplification of the PfMDR1 locus in DD2 and a large deletion in the CLAG3.2 gene in GB4, and putative novel CNV regions. When compared to the recently available FREEC and cn.MOPS approaches, our findings were more concordant with putative hits from the highest quality array data for the 7G8 and GB4 isolates. These promising results motivate the application of the methodology to a larger collection of *P. falciparum* samples of different origins.

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THE EVOLUTION AND GENETIC DIVERSITY OF THE CIRCUMSPOROZOITE PROTEIN (CSP) IN *PLASMODIUM SPP.*

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The Circumsporozoite Protein (CSP) is the predominant constituent of the sporozoite surface and plays several fundamental roles, including in the development of the oocyst and the first merozoites in the liver during the pre-erythrocytic cycle. In this investigation, we amplified, cloned and sequenced the CSP gene from several *Plasmodium* species. We separate them into two groups, the Laverania clade that includes *P. falciparum* and related species (5 species) and the *Plasmodium* clade that includes *P. vivax* and related species from monkeys in Africa and Asia (11 species). The alignment and phylogenetic analyses of CSP sequences were performed using only the conserved N and C-terminal regions of the gene. Although the CSP phylogeny follows the trend evidenced by other *loci* (e.g. mtDNA), we found distinctive patterns among *Plasmodium* lineages. From all the species studied, only the two African Cercopithecidae parasites, *P. gonderi* and one from mandrills, lack region I sequence (KLKQP), which plays a critical role in the processing of the CSP in the mammalian host due to its high affinity to heparin sulfate on the surface of liver cells or hepatocytes. We also found that the central tandem repeat region exhibited extensive genetic diversity even among closely related species, or within a single species, in terms of the number of repeats with diverging motifs in many malarial parasites (e.g. P. inui). However, the Laverania clade shows strong conservation in the basic tandem motifs of the CS protein. The asparagine rich motifs, previously reported, PNAN and PNVD, are present not only in *P. falciparum* but also in its closely related species. There are, however, several new motifs found in low frequency such as PNADPN in *P. billcollinsi* and *P. billbrayi* and PNVN in *P. reichenowi*. Finally, we found contrasting patterns of selection acting on the N and C-terminal regions in the two major human parasites. Thus, our findings indicate that the CS protein evolved under different constrains in the *P. falciparum* and *P. vivax* clades.

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MALARIAL PARASITE DIVERSITY IN CHIMPANZEES: COMPARATIVE APPROACHES TO ASCERTAIN THE EVOLUTION OF *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum, the agent of the most lethal form of human malaria, shares its most recent common ancestor with parasites found in African apes. Here we studied *Plasmodium* lineages found in chimpanzees (*Pan troglodytes*) and explored their recent evolutionary history. We also studied genes orthologous to two currently considered in antimalarial vaccines: merozoite surface protein 2 (MSP2) and the DBL-1 domain from var2CSA gene. In order to study malaria parasite diversity, we screened 74 blood samples of chimpanzees that were collected in the Democratic Republic of Congo in 2010. We amplified, cloned and sequenced the parasites' mitochondrial genomes (mtDNA), the chloroquine resistance transporter (Pfcrt), merozoite protein 2 (MSP2), and the DBL-1 domain from the var2CSA gene, for all positive samples. We found nine positive chimpanzees (12.2%). Four of the nine positive samples were identified as *P. falciparum*, two as *P. reichenowi*-like, one as *P. gaboni*, and two as *P. malariae*. All *P. falciparum* samples were resistant to chloroquine suggesting that they acquired such infections from humans. Time estimates based on this expanded data set support that the evolutionary events leading to *P. falciparum* include an extended period of co-evolution with hominids. Our study indicates that the proposed species: *P. gaboni, P. billrayi*, and *P. billcollinsi* still hold in this extended data set. In the case of msp2, we provide evidence of the recent origin of the two major groups of MSP2 alleles and conclude that they originated after the *P. reichenowi - P. falciparum* split. The var2CSA gene was also found in relatively divergent chimpanzee malaria lineages. This gene accumulated extraordinary genetic polymorphism after the *P. reichenowi* and *P. falciparum* split. These examples support the notion that comparative genomic approaches among *P. falciparum* and its related species will be of great value in understanding the evolution of proteins that are important in parasite invasion of the human red blood cell, as well as those involved in malaria pathogenesis.

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EVIDENCE OF CLONAL *PLASMODIUM FALCIPARUM* POPULATIONS IN EASTERN PANAMA

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Elimination of malaria from Meso-America urges for the characterization of circulating field strains of *Plasmodium* parasites from the region. In this study we examined 41 DNA samples obtained from *P. falciparum* field isolates collected during an epidemic that occurred in Panama in 2003-2008, and characterized their genetic diversity and relatedness using a SNP-Based Molecular Barcode assay. Principal Component Analysis of the barcode data let us to characterize, map and group the isolates into three distinct clonal sub-populations ($p = 0.0001$), two from the pacific watershed of the isthmus identified as Madugandi and Darien that

clustered together as one clonal group, while the other belonging to the Guna Yala group clustered into two distinct clonal sub-populations. The identical barcodes observed in three subpopulations of *P. falciparum* field isolates indicates that these subpopulations are highly related and could be the result of clonal propagation or epidemic expansion, or both. These findings support the hypothesis that highly related parasite populations are evident in low transmission settings, such as observed in Panama. We anticipate that use of genomic tools such as the molecular barcode will detect changes in malaria parasite population structure that occur as malaria-reducing strategies are implemented regionally to lower transmission. Such tools allow tracking of specific parasite types and we anticipate that these data will help in the planning, design and implementation of malaria elimination programs tailored for the southern region of Meso-America.

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DRUG INTERACTION EVALUATION OF PYRONARIDINE/ARTESUNATE AND METOPROLOL AND RE-DOSING EVALUATION OF PYRONARIDINE/ARTESUNATE IN HEALTHY VOLUNTEERS

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Pyronaridine/artesunate (PA) is an ACT indicated for treatment of uncomplicated *falciparum* and *vivax* malaria in children and adults. We conducted an open-label randomized study 1) to evaluate for a drug-drug interaction between PA and the CYP2D6 substrate metoprolol, 2) to assess for any effect of PA re-dosing after 60 or 90 days on the pharmacokinetics of pyronaridine, and 3) to explore any relationships between CYP2D6 metabolizer status and pyronaridine pharmacokinetic parameters. Healthy adult subjects were randomized to Arm A or Arm B. Arm A subjects were administered 100 mg metoprolol tartrate alone in the first period, three daily doses of PA with 100 mg metoprolol tartrate with the third PA daily dose in the second period, and three daily doses of PA alone 90 days later in a third period. Arm B subjects received the three day PA regimen alone in the first period, with re-dosing of this three day regimen occurring after 60 days in the second period. Non-compartmental pharmacokinetic parameters were computed for metoprolol, its metabolite alpha-hydroxymetoprolol, and pyronaridine; pyronaridine parameters were based on concentrations obtained during the third day of any given PA dosing period. Pharmacokinetic analysis indicated that co-administration of metoprolol and PA was associated with an average 47.93% increase in metoprolol maximum concentration and a 25.60% increase in metoprolol $AUC_{0\text{-}t}$; these increases most likely resulted from pyronaridine-mediated CYP2D6 inhibition. No interaction effect of metoprolol on pyronaridine was apparent. Furthermore, the pharmacokinetic re-dosing analysis did not suggest a relevant effect of re-dosing after either 60 or 90 days on pyronaridine pharmacokinetics. Finally, a comparative evaluation of pyronaridine pharmacokinetics in poor, intermediate, extensive, and ultra-rapid CYP2D6 metabolizers did not reveal any clear pattern of pharmacokinetic differences.

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MALARIA THERAPY IN LAGOS, NIGERIA: UPSURGE IN CHLOROQUINE USE COMPARED WITH ACTS

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Artemisinin based combination therapies (ACTs) were adopted in 2005 as standard treatment for uncomplicated malaria in Nigeria to counter resistance of *Plasmodium* to antimalarial drugs such as chloroquine and sulphadoxine/pyrimethamine used previously. Periodic evaluation of the use of ACTs is needed for intervention and control of resistance to this medication. Lagos State in Nigeria contains a potential megacity and a significant proportion of dwellers in the state are treated for malaria in private hospitals. Retrospective studies of 1827 and 1184 prescriptions in 23 private hospitals were done in 2007 and 2012 respectively. Hospitals were selected from 20 local government Areas in Lagos State by multistage sampling method. Use of chloroquine (CQ) constituted 10.1% of anti-malarials prescriptions in 2012 compared with 2.7% in the 2007 study. In contrast, ACTs prescription decreased from 81.1% in 2007 to 53.3% in 2012. Prescriptions which contained only 1 antimalarial agent rose from 10.4% to 30.1% in 2007 and 2012 studies respectively. Triple combinations of antimalarials as ACTs + one antimalarial agent was noted for the first time in the more recent study, these constituted 9.1% of prescriptions. Urgent intervention is needed to promote rational treatment of malaria in Lagos State.

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WWARN *IN VITRO* PILOT PROJECT: HOW TO REDUCE VARIABILITY FOR *IN VITRO* SUSCEPTIBILITY TESTING OF ANTIMALARIAL DRUGS

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In vitro susceptibility of antimalarial drugs is an important part of resistance surveillance as susceptibility can be assessed without immunity or pharmacokinetic confounders, and the isolated effect of the partner drug in an artemisinin-based combination therapy can be determined. As different methodologies are used between different laboratories, lack of comparability is a challenge for in the interpretation of *in vitro* data. In this pilot project we addressed this issue by assessing intra- and interlaboratory variability and determine factors of variability for *in vitro* testing. Twenty participating laboratories tested the *in vitro* susceptibility of the reference clones 3D7 and W2 to chloroquine, mefloquine, desethylamodiaquine and dihydroartemisinin. Testing was performed with each laboratory's established methodology and WWARN provided the following measures of standardization: 1) genetic validation of the reference clones by microsatellites and pfmdr1 copy number, 2) validated, pre-weighed drugs supplied from the WWARN Reference Material Scheme, and 3) reproducible data analysis using the WWARN *In Vitro* Analysis and Reporting Tool (IVART). In preliminary analysis large interlaboratory variability was demonstrated, especially for dihydroartemisinin. Laboratories using two read-out methods showed low variability between the methods, motivating exploration of the effects of culture conditions as well as read-out methods on variability. Improved understanding of the factors determining variability will be used to make recommendations on strategies to reduce variability to improve comparability and standardization of *in vitro* testing, within and between laboratories. This in turn can result in more reliable and reproducible data and increase usefulness of *in vitro* data for tracking antimalarial drug resistance and validating molecular markers.

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BENZOXABOROLE ANTIMALARIAL AGENTS: STRUCTURE-ACTIVITY RELATIONSHIP (SAR) OF 6-SUBSTITUTED-1,3-DIHYDRO-1-HYDROXY-2,1-BENZOXABOROLES

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Emerging resistance to the frontline antimalarial drug artemisinin demonstrates the need for new drugs with different structures and targets. To ensure coverage of the widest chemical space, new libraries containing unique chemical scaffolds should be screened. Anacor's library of boron-containing compounds is one such library and has been shown to be a rich source of compounds active against pathogens that cause neglected diseases. For example, SCYX-7158 (AN5568) is in phase I human trials for the treatment of human African trypanosomiasis. Screening of the Anacor boron library has yielded multiple families of benzoxaboroles with activity against malaria parasites. Among these compounds, 6-aryl and 6-aryloxy-1,3-dihydro-1-hydroxy-2,1-benzoxaboroles were identified in screens against cultured malaria parasites, and additional compounds were designed and synthesized. With this process potencies against W2 and 3D7 strain *Plasmodium falciparum* were improved 2500-fold (IC_{50} 1.0 μM to 0.4 nM). Based on available data, structure-activity relationships show that the substituent at the benzoxaborole 7-position has significant impact on antimalarial potency. The results of a more detailed SAR investigation of these benzoxaborole compounds will be presented.

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A RANDOMIZED STUDY TO ASSESS THE EFFICACY AND TOLERABILITY OF THREE ARTEMISININ BASED COMBINATION THERAPIES FOR THE TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA IN THE DEMOCRATIC REPUBLIC OF CONGO

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With increasing resistance in Africa to a range of antimalarial drugs, new treatment options for *Plasmodium falciparum* malaria are urgently needed. We tested safety, tolerability and efficacy of the artemisinin-based combination treatment dihydroartemisinin-piperaquine (DP) in children with uncomplicated *P. falciparum* malaria in the Democratic Republic of Congo (DRC). We compared DP to amodiaquine-artesunate (AS), first-line treatment in DRC since 2006 and artemether-lumefantrine (AL) recently added to the first-line.An open label, individually randomized, controlled trial was carried out in 2011-12 in a malaria endemic sector of Kinshasa. Children aged 3 to 59 months with uncomplicated *P. falciparum* malaria were randomly allocated to AS, AL or DP. Children were hospitalized for three days, given supervised treatment and followed-up weekly for 42 days. The primary endpoint was efficacy defined as the *P. falciparum* PCR-adjusted cure rate assessed by day 42.Six hundred and eighty four patients were recruited. The median *parasitemia* on admission was 32,000 parasites/μL (range 2,040 to 199,960) and 6% of patients had >175,000 parasites/μL. The mean time for the log *parasitemia* to decrease by 50% was 2.69 hours (SD 0.84; range 2.56 to7.29) and similar between arms (p=0.18). All patients cleared the infection completely within 72 hours of admission. The PCR unadjusted cure rates by day 42 were AS=73.5%, AL=70.6% and DPQ=86.8% (p=0.001). Early treatment failure occurred in three patients (0.5%), one in each arm. The PCR adjusted cure rates were AS=93.8% AL=93.1%, DP=94.8% (p=0.76). The mean PCV at admission was 30.2% (SD 4.9) and an overall mean reduction of 10.1% (SD 8.3) was observed with 6.0% of patients experiencing a reduction of >25% of the admission value, with no difference between arms (p=0.65). Ten patients required a blood transfusion during hospitalization. The regimens were well tolerated and there were no drug-related serious adverse event related to the treatments. All combinations were equally effective in treating the disease with a favourable safety and tolerability profile. DP provided greater protection from new episodes of malaria during the 42 days follow-up compared to either AL or AS (Trial Registration ISRCTN20984426).

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EVALUATION OF THE QUALITY OF MALARIA TREATMENT AND OF MALARIA IN PREGNANCY IN HEALTH FACILITIES IN MALI

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In Mali, malaria is the major cause of morbidity and mortality especially for children under 5 and pregnant women. Since 2007 Mali has benefited from the President's Malaria Initiative (PMI) funding. In order to assess the impact of this support a cross-sectional study to evaluate malaria case management and antenatal care in health facilities was undertaken. Three methods of gathering information were used: 1) Provider observations during curative or antenatal care, 2) Re-examination of the patients whose consultation (curative and ANC) was previously observed, 3) Interviews of providers, patients and health facility managers. ACT were available for free for children under five/pregnant women or for sale for other ages in all of our sample of health facilities, the vast majority of health facilities did not have mosquito nets for distribution. 57.8% of providers reported having participated in a course of formal training on malaria case management using ACTs. For uncomplicated malaria in children under 5, 79.3% of diagnoses were without diagnostic error. However, 52.6% of patients with suspected malaria were not given a diagnostic test. All malaria prescriptions were analyzed. For uncomplicated malaria in children under 5, the correct information for the number of days was given 12 of 13 times, every time for the number of doses per day, and 11 of 13 times for the name of the drug and definition of the dose. For severe case management in children under 5, 6 out of 10 providers gave the correct number of treatments per day, and 2 out of 10 prescribed the correct number of days. The results are even poorer for patients over five years: 11 of 12 prescriptions gave a wrong number of days of treatment and 6 the incorrect number of doses per day. In conclusion, management of uncomplicated malaria is satisfactory, but the management of severe malaria is weak. National treatment guidelines requiring laboratory confirmation of all malaria cases are not followed in half of the cases, confirmation is particularly low for suspected cases in pregnant women.

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CLINICAL DETERMINANTS FOR EARLY PARASITOLOGICAL RESPONSE IN PATIENTS DIAGNOSED WITH UNCOMPLICATED MALARIA IN AFRICA TREATED WITH ARTEMISININ COMBINATION THERAPIES: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Slow clinical and parasitological response after artemisinin therapy for uncomplicated *Plasmodium falciparum* malaria has been reported in the Mekong region. Further spread of these parasites poses a major global public health threat, especially in sub-Saharan Africa where the

disease burden is greatest. In this pooled analysis, the geographical and temporal trends of early parasitological response following artemisinin-based combination therapy (ACT) treatment in African clinical trials. The cofactors affecting early and late treatment outcome were investigated. Individual patient data from efficacy trials were shared with the Worldwide Antimalarial Resistance Network (WWARN) and pooled using a standardised methodology. Data from 52 clinical efficacy studies (N=19,078) conducted in Africa (2002-2011) of Artemether-Lumefantrine (AL, n=9,377), Artesunate-Amodiaquine (ASAQ, n=6,167) and Dihydroartemisinin-Piperaquine (DP, n=3,534) were included in the analyses. The risk of remaining parasitaemic increased on day 1 [AOR: 1.5, 95% CI: 1.3-1.7, P<0.001], but decreased on day 2 [AOR: 0.85, 95% CI: 0.74-0.98, P=0.02] and day 3 [AOR: 0.7, 95% CI: 0.5-0.9, P=0.01], reflecting probably a reduction in population-level clinical immunity. Baseline parasitaemia at enrolment was the most important risk factor affecting parasitological response at day 1 [AOR: 1.75, 95% CI: 1.7-1.8, P<0.001], day 2 [AOR: 1.4, 95% CI: 1.3-1.5, P<0.001] and day 3 [AOR: 1.3, 95% CI: 1.1-1.5, P<0.01]. Patients who remained parasitaemic on any of the first three days were at substantially greater risk of subsequent recrudescent infection: AHR=1.6 [95% CI: 1.1-2.2, p=0.01] on day 1, 1.6 [95% CI: 1.1-2.4, p=0.02] on day 2 and 3.5 [95% CI: 1.7-7.1, p<0.001] on day 3. Although a delay in early parasite clearance was associated with treatment failure, there was no evidence from the data examined that the overall speed of clearance is declining in Africa. Drug resistance surveillance needs to be vigilant and strengthened to limit geographical and temporal gaps in data.

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TREATMENT OF SEVERE MALARIA - AN OPERATIONAL COMPARISON BETWEEN QUININE AND ARTESUNATE FOR THE TREATMENT OF SEVERE MALARIA IN SEVEN HEALTH FACILITIES IN THE DEMOCRATIC REPUBLIC OF THE CONGO - "MATIAS"

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About 8 million cases of severe malaria occur each year, with a particularly heavy toll on pregnant women and children. The Democratic Republic of the Congo (DRC) is the second most malarious country in the world (after Nigeria) and has the highest severe malaria burden. Recent trials comparing injectable artesunate with quinine have demonstrated relative mortality reductions of 34.7% in adults and 22.5% in children, with lower side effects. In 2011, WHO recommended injectable artesunate as the preferred option for treatment of severe malaria. In early 2012 the National Malaria Control Program (PNLP) of the DRC adopted the WHO guidelines and changed the policy for treatment of severe malaria in children and adults to injectable artesunate. However, the nationwide rollout is a complex undertaking, requiring many operational and clinical adaptations. To provide information and support this process in a country marred by technical and logistical challenges, a limited scope implementation study was designed. The study comprises four key components: a) clinical safety and efficacy assessment on the basis of routine patient information; b) time-and-motion study to study operational parameters; c) feasibility and acceptability assessments through provider and patient/caretaker questionnaires; d) financial cost analysis. The study is conducted in seven health facilities in three rural and one urban health zones of the DRC. In a first phase 410 patients were treated with injectable quinine. After a transition and training phase 350 patients will be enrolled until the end of June 2013 and treated with injectable artesunate. Following recent reports on hemolytic anemia being potentially related to the use of injectable artesunate and CDC's January 2013 recommendation for an expanded follow-up phase of patients of 28 days, the study was amended accordingly. Hemoglobin levels are now measured at days 7, 14, 21 and 28 after treatment. The overall fatality rate in the population treated with quinine was 3.4%; the results for injectable artesunate are expected for September 2013.

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IMPACT OF MULTIPLE DOSES OF INTERMITTENT PREVENTIVE TREATMENT REGIMENS IN PREGNANT WOMEN ON BIRTH WEIGHT IN SOUTHERN PROVINCE, ZAMBIA

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Malaria in pregnancy (MiP) causes negative neonatal outcomes, such as low birth weight (LBW), a significant contributor to infant mortality. The Zambian Ministry of Health recommends 3 doses of sulfadoxine-pyrimethamine (SP) as intermittent preventive therapy in pregnancy (IPTp) to prevent MiP. The new World Health Organization (WHO) guidelines now recommend monthly SP IPTp as a replacement for the previously recommended 2-dose regimen, after a meta-analysis demonstrated the superiority of 3 or more doses in preventing LBW and other complications of MiP. This analysis compares the impact of the current IPTp-SP guidelines (3 doses or more) to the prior guidelines (2 doses or more) in reducing LBW in Southern Province, Zambia. We hypothesized that the 3-dose group will have a lower percentage of LBW newborns and a higher mean birth weight. We performed a secondary analysis using a subset of participants (n=14,414) enrolled in the Zambia Chlorhexidine Application Trial (ZamCAT) in Southern Province, Zambia. Using ANOVA and multivariate linear regressions, we assessed the impact of the number of SP doses (0, 1,2,3,4+) on birth weight. Logistic regression models were used to compare the proportion of LBW infants between groups, and to examine the impact of the previous SP IPTp guidelines (2 doses versus no SP) compared to the current guidelines (3+ doses versus no SP) on LBW. We found an increase in birth weight and a decrease in the proportion of LBW infants with increasing number of SP doses (p <0.0001). Treatment with the prior WHO guidelines provides a trend towards a protective effect against LBW among premature newborns (OR=0.77, 95% CI 0.58, 1.03) but not among newborns of normal gestational age (OR= 1.15, 95%CI 0.9,1.46). A stronger protective effect was observed with the new guidelines, particularly among premature newborns (OR=0.64, 95%CI 0.50, 0.80). The new IPTp recommendations may help reduce the risk of LBW among premature newborns, and thus may serve to decrease their risk of early mortality even in the context of high levels of SP resistance.

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ASSESSING THE EFFECT OF THE RECOMMENDED ARTEMETHER-LUMEFANTRINE DOSING REGIMEN ON THE RISK OF TREATMENT FAILURE IN PATIENTS DIAGNOSED WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Artemether-lumefantrine (AL) is the first line antimalarial treatment in 49 countries, administered according to four weight bands. Patients at the margins of these bands can receive significant deviation from the target dose. To assess the impact of weight adjusted (mg/kg) dose variations in therapeutic efficacy, individual patient data were shared with the WorldWide Antimalarial Resistance Network (WWARN) and collated using standardised methodology. Risk factors associated with recrudescence were evaluated using Cox's regression model with shared frailty on study sites. Data from 14,986 patients (65 efficacy studies between 1996 and 2011) with uncomplicated *Plasmodium falciparum* malaria were included in the analyses (Africa: 12,179, Asia: 2,648, South America: 159). A total of 320 Polymerase Chain Reaction (PCR)-confirmed recrudescent infections were reported. The median (IQR) total lumefantrine dose was 68.6 mg/kg (57.6-80.0), with children <1 year receiving the greatest dose [87.8 mg/kg (75.6-98.6)], compared to those between 1 and 5 years [67.9 mg/kg (59.0-80.0)], 5 and 12 years [74.5 mg/kg (65.5-83.1)] and ≥12 years [57.6 mg/kg (49.9-65.5)]. The median (IQR) mg/kg dose of lumefantrine in patients failing treatment was 70.6 mg/kg (57.6-80.0), and did not differ significantly from those who were cured [68.6 mg/kg (57.6-80)]. In a multivariate model the log of the baseline parasitaemia and young age (1 to 5 years) were both significant risk factors for recrudescent infections (AHR=1.14 [95 CI%: 1.04-1.24, p=0.003] and 2.27 [95 % CI: 1.43-3.58, p<0.001]) respectively, accounting for 41% of all treatment failures. Treatment supervision and co-administration without a fatty meal were not associated with increased risk of recrudescence. In this pooled clinical analysis, the mg/kg dose of lumefantrine administered was not correlated with treatment failure suggesting that current dosing strategies of AL are robust.

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SEASONAL MALARIA CHEMOPREVENTION IN SENEGAL: FROM RESEARCH TO POLICY

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Seasonal malaria chemoprevention (SMC) is a new strategy for malaria control in children. Studies conducted in Sahelian and sub-Sahelian Africa have shown that SMC is highly effective, safe and can be delivered at large scale with high coverage in areas where malaria is seasonal. In 2011, through a cluster randomized trial in southern Senegal, we investigated the effectiveness of SMC combined with community case management for preventing the burden of malaria in the study area. Other objectives of the project included an assessment of the feasibility and tolerability of SMC in older siblings (5-9 years old) and for a period of 5 months. The primary endpoint was the incidence of malaria (fever or history of fever with a positive RDT). Preliminary results show a 82 % protective efficacy of SMC. A positive impact on severe anemia and *parasitemia* has also been found. SMC is now adopted as national policy in Senegal and will be implemented as of August this year in 4 regions (Tambacounda, Kédougou, Kolda, Velingara) all located in the southern part of the country totaling around 550000 children under 10 years of age. In these regions, the clinical attack rate of malaria is greater than 0.1 attack per transmission season in children under 10 years of age. The impact of SMC on malaria morbidity and mortality will be evaluated and effects on natural acquisition of immunity explored using a case control approach. Prevalence of molecular markers of resistance to SMC drugs will be measured in blood samples in used RDTs. In the west African sub region 9 countries with Senegal have started the process to incorporate SMC among their malaria control strategies. A partnership between LSHTM, UCAD, WHO and WARN has been set up to help these countries to elaborate their implementation plan.

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HEMOGLOBIN LOSS AND ITS ASSOCIATION WITH PROTECTION AT RELATIVELY LOW PARASITEMIA IS INFLUENCED BY A HOST GENETIC FACTOR IN SEMI-IMMUNE MICE INFECTED WITH *PLASMODIUM BERGHEI* ANKA

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Human studies and our previous animal studies have shown that there are individual responses towards malarial infection even under the same malarial transmission intensity. These studies also show that individuals with low haemoglobin (Hb) are protected. We, therefore hypothesize that host genetic factors is/are influencing these differences affecting the extent to which Hb is synthesized during malaria infection. In testing this hypothesis we crossed two mice strains (Balb/c of low parasitaemia) and CBA (moderately high parasitaemic) to get the progeny, called the F1. Balb/c (8), CBA (8) and F1 (12) were taken through 6-7 cycles of infection (with *Plasmodium berghei* ANKA) and treatment (with chloroquine/pyremethamine) to generate semi-immune status. Parasitaemia and haematological parameters were monitored. Kinetics of antibody production, cytokine levels (in serum and cultured supernatant of stimulated spleen cells) and CD4+CD25+T regulatory cells were evaluated by ELISA, bead-based multiplex assay kit and FACs respectively; at days 0, 16, 28 for Balb/c and F1, and days 0, 8 and 12 for CBA. Similar survival (>70%), mean %Hb loss (45%) and mean parasitaemia (5%) was observed in Balb/c and F1, while 0% survival, mean %Hb loss (80%) and mean parasitaemia of 15% was observed in CBA. IgG subtypes were two times higher in Balb/c and F1 than CBA. While IL1a, IL4, IL10, IL12a, IFNγ and TNFα were similar in the three mice strains, IL17 was 4.5 times higher in Balb/c and F1 than CBA. Increasing trend of cytokines levels was observed in CBA whilst a maximum cytokine level was observed at D16 (point at which recovery from parasitaemia occurs, with lowest Hb) in the Balb/c and F1. CD4+CD25+ Treg cells in CBA were similar on days measured, but lower than those of Balb/c and F1. In conclusion, innate mechanism of Hb loss in controlling parasitaemia level, hence survival is similar in Balb/c and F1. A genetic factor controlling this Hb loss in Balb/c is passed onto the F1 progeny.

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HEMOGLOBIN-α 2-PROMOTER VARIATION INFLUENCES SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM*-ASSOCIATED ANEMIA

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Severe malarial anemia [SMA, hemoglobin (Hb) less than 5.0g/dL], due to infection with *Plasmodium falciparum*, is a leading cause of morbidity and mortality in African children. The underlying genotypic traits that influence SMA have not been fully elucidated. Current findings using

high-throughput genotyping [Human BeadChip' (2.45x106 markers)] and global gene expression arrays [HumanHT-12 v4 BeadChip (47,231 probes)] to investigate a pediatric population in Kenya (3-36mos) identified a significant association between several genetic variants and SMA. Whole genome genotyping in a subset (n=100) of children with *falciparum* malaria (non-SMA vs. SMA) revealed >2 copies of hemoglobin-α2 (HBA2) and several polymorphic variants in the promoter region of children with SMA. In addition, global gene expression profiling showed a 1.81-fold change in HBA2 in the SMA group. HBA2 codes for one of the two α-chains of hemoglobin. Previous studies demonstrated that similar polymorphisms generate hemoglobinopathies that protect against *P. vivax* and *P. falciparum* infections in pediatric cohorts. To extend and confirm the whole genome findings, we performed in silico analysis for HBA2 and identified two (potentially) functional SNPs (rs1203833 and rs2974771) that were then genotyped in a larger cohort (n=739), followed by construction of haplotypes. Binary logistic regression analysis, controlling for covariates (age, gender, G6PD, HIV-1, *bacteremia*, α-thalassemia, and HbAS status) revealed that carriage of the TG haplotype (-1789T/-4314G) increased susceptibility to SMA (OR: 1.61, 95%CI: 1.11-2.32, P<0.05). In addition, a reduced erythropoietic response (RPI<2) was observed in carriers of the AG (-4314A/G; OR: 0.65, 95%CI: 0.44-0.96, P<0.05) and TT (-1789C/T; OR: 0.50, 95%CI: 0.29-0.85, P<0.05) genotypes, while carriage of the CA (-1789C/-4314A) haplotype was associated with enhanced erythroid production (OR: 3.11, 95%CI: 1.34-7.22, P<0.001). Taken together, these results demonstrate that variation in HBA2 is associated with susceptibility to SMA and altered erythropoietic responses.

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A DELETION OF 3.7 KILOBASES OF DNA IN THE ALPHA GLOBIN GENE (-α3.7 THALASSEMIA) PROTECTS CHILDREN AGAINST SEVERE MALARIAL ANEMIA IN WESTERN KENYA

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Alpha (α)+ thalassemias (α-thal) result from deletions of one or both of the duplicated α-globin genes and/or inactivating mutations. In sub-Saharan Africa, the most common deletions are the -α3.7, often presenting as heterozygous (-α3.7/αα) and homozygous (-α3.7/-α3.7) forms. Although both heterozygous and homozygous α+ thals confer protection against fatal *Plasmodium falciparum*malaria, their effect on severe malarial anemia (SMA; Hb less than 5.0g/dL; with any density *parasitemia*) as the primary clinical outcome of severe disease has not been determined. As such, children (less than 3 months; n=990) living in a holoendemic malaria transmission region of western Kenya were recruited into the study and grouped into three categories: aparasitemic controls (AC; n=239), non-SMA (Hb less than 5.0g/dL; n=611), and SMA (Hb less than 5.0g/dL; n=140). a3.7 thal deletion variants were genotyped and their effect(s) on clinical outcomes was investigated. The proportion of deletion variants .distributed across the groups was: aa/aa, 0.221 (AC), 0.622 (non-SMA), and 0.156 (SMA); -a3.7/aa, 0.242(AC), 0.636 (non-SMA), and 0.121 (SMA); and a3.7/-a3.7, 0.277 (AC), 0.580 (non-SMA), and 0.143 (SMA). The distribution frequency across the clinical groups were comparable (P=0.370). Multinomial logistic regression analysis modeling, controlling for confounders, indicated that homozygous carrier of the -a3.7/-a3.7 variants were significantly protected against both non-SMA [OR=0.615 (95%CI 0.405-0.935) P=0.023] and SMA [OR=0.339 (95%CI 0.138-0.834); P=0.019]. In addition, carriage of the -a/aa genotypes partially protected against non-SMA [OR=0.807 (95%CI 0.553-1.073); P=0.065] and SMA [OR=0.566 (95%CI 0.242-1.171); P=0.091]. Taken together, these results demonstrate that α+-thal deletions confer a protective advantage against malarial anemia in regions of holoendemic malaria transmission.

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POTENTIAL *PLASMODIUM* SPECIES-SPECIFIC VULNERABILITIES TO TRANSMISSION BLOCKING DUE TO HOST IMMUNE RESPONSES: INSIGHTS FROM MATHEMATICAL MODELING

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Experiments involving transmission of avian and rodent malarias show that host factors can attack the gamete forms of the parasites in the midgut of mosquito vectors, an effect known as transmission blocking. In addition, some human patients infected with either *Plasmodium falciparum* or *P. vivax* develop antibodies against the gamete forms of those species. However, a 1977 field study in Gambia suggested that host immune responses directed against the within-host gametocyte forms (precursors of the gametes, before uptake by mosquitoes) had little effect on *P. falciparum* transmission. On the other hand, a 2008 field study (again in Gambia) showed that patients who had antibodies to surface antigens of transmissible *P. falciparum* gametocytes cleared gametocytes earlier than those who did not. Using theory from population biology, we developed mathematical models of the within-host asexual forms and gametocytes interacting with host innate and antibody immune responses. Models were tailored for the specific life cycles of *P. falciparum* and *P. vivax*, and for wide ranges in host immune capacity to detect and clear parasites. We show that for both *Plasmodium* species, for the same ability to detect and clear a targeted parasite stage, antibodies to the immature (pre-transmissible) gametocytes would be more effective in reducing the density of transmissible gametocytes than antibodies directly targeting the transmissible forms. But we also found that due to the longer time needed for gametocytes of *P. falciparum* to mature, this species is much more vulnerable than *P. vivax* to transmission blocking by host antibodies to the immature gametocytes. Since field studies indicate that transmissible gametocyte density in malaria patients infected with either species are the same, on average, our results suggest that *P. falciparum* has evolved mechanisms to evade or suppress host immune responses that can effectively eliminate immature gametocytes.

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CYTOKINES AND ANTIBODIES LEVELS AND PREVALENCE OF CONGENITAL AND NEONATAL MALARIA IN MALI

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At the Pediatric ward of the National Teaching Hospital Gabriel Toure, which is the main tertiary Pediatric reference hospital of the country, neonatal mortality was 30%, 36.7% and 61% in 1997, 1999 and 2000, respectively. Because of scarce resources, no exact etiology of these deaths is known. However, based on clinical signs, the great majority of these illnesses are categorized as of infectious origin. Pediatricians use their clinical judgment to prescribe antibiotics and other treatments without laboratory evidence of etiology. We proposed to test the hypothesis that the prevalence of congenital and/or acquired malaria is negligible in new born infants in Mali. We used sensitive molecular biology and biochemical methods to measure the prevalence of malaria in preterm infants and in neonates admitted to the Pediatric ward of Hospital Gabriel Toure. We found that all 300 infants were negative for malaria *parasitemia* using both microscopy and PCR. The OptiMal IT was positive for *P. falciparum* in 3 infants (1%). Among the 146 mothers included in the study we found that 0 (0%), 1 (0.7%) and 10 (6.8%) were positive for malaria parasites using microscopy, OptiMal IT and PCR, respectively. Cytokine analyses showed that neonates had a strong anti-inflammatory response, significantly higher than their mothers (p< 0.05). The response was significantly higher than that of PCR (+) mothers for IL2 and IFN-gamma. Similarly, PCR (-) mothers had higher levels of MSP3 and GLURP

antibodies. Our data suggest that there is no malaria in congenital and neonate population. Immunity factors may play a key role in the protection of congenital and neonate infants.

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DOES LIGAND BINDING ALTER THE IMMUNOGENICITY OF *PLASMODIUM FALCIPARUM* AMA1?

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Apical membrane antigen 1 (AMA1) has been considered a leading malaria vaccine candidate but polymorphisms in AMA1 limit its efficacy. In a recent Phase 2 trial in Mali an AMA1 vaccine had significant efficacy only against parasites expressing a form of AMA1 related to that in the vaccine1. Combining multiple allelic forms of AMA1 in a vaccine may overcome this problem by directing antibody responses to conserved epitopes but this would increase the cost of an AMA1 vaccine. A possible alternative approach was suggested by the observations that the most polymorphic region (C1-L) on the surface of AMA1 is adjacent to the RON2 binding site on AMA1, and when a ligand binds into this hydrophobic pocket, there is a significant rearrangement of the conserved domain II loop. Groups of mice were immunized with three different AMA1-peptide complexes; two peptides (R1 and R3) were isolated from a phage-displayed peptide library and the third (RON2L) is a segment of the natural ligand of AMA1. Sera from the mice were analysed by direct and inhibition ELISAs using a variety of forms of AMA1, including chimeric constructs displaying regions of *P. falciparum* AMA1 on a *P. berghei* AMA1 background. Although the complexes induced good responses there was little evidence that the antibodies were more cross-reactive than those induced by uncomplexed AMA1. The results of this preliminary experiment are not encouraging but we will explore this approach further using immunogens in which the peptide ligands have been chemically cross-linked to AMA1.

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ANTIBODIES THAT PROMOTE PHAGOCYTOSIS OF *PLASMODIUM FALCIPARUM* MEROZOITES ARE ASSOCIATED WITH PROTECTION AGAINST MALARIA AND CAN BE INDUCED BY HUMAN VACCINATION

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Malaria illness develops during the blood-stage infection when the merozoites form of the parasite infect human erythrocytes and replicate inside them. Merozoite antigens are important targets of antibodies that are thought to be the key mediators of protective immunity, and merozoite antigens have long been regarded as promising vaccine candidates. The protective mechanisms of antibodies against merozoite antigens are not well understood, but may include opsonization of merozoites for phagocytic clearance. Currently, there is a lack of assays to measure functional antibody activity in studies of acquired and vaccine-induced immunity in humans. To address these questions, we developed a high-throughput assay to quantify antibody-mediated phagocytosis using a human monocyte cell line and purified *P. falciparum* merozoites. We used this assay to assess the opsonic activity of antibodies in cohort studies of children and adults in Kenya, and a phase 1 vaccine trial of a major merozoite surface antigen. We found that antibodies that promote opsonic phagocytosis were acquired with increasing age and exposure to malaria, and broadly correlated with IgG levels to merozoite antigens measured by ELISA. Importantly, high levels of opsonic phagocytosis activity among children were prospectively associated with a decreased risk of malaria, suggesting a role in protection. Human immunization with recombinant merozoite surface protein 2 (MSP2), an abundant protein on the merozoite surface, generated cytophilic antibodies, IgG1 and IgG3, that bound the merozoite surface and promoted opsonic phagocytosis. Our findings suggest that opsonic phagocytosis of merozoites may be an important mechanism by which antibodies to merozoite antigens contribute to protective immunity to malaria and and support the further development of merozoite surface proteins as potential malaria vaccine antigens. Furthermore, these studies have established a high-throughput assay to measure the functional activity of antibodies to merozoites to investigate acquired and vaccine-induced human immunity.

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IMMUNOLOGICAL CORRELATES OF REINFECTION IMMUNITY IN MURINE MALARIA *PLASMODIUM YOELII*

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Immunological mediators of reinfection immunity in malaria are poorly understood. We studied the immune correlates of immunity during reinfection with non-lethal *Plasmodium yoelii* 17XNL parasites in BALB/c mice that had once cleared their infection with the homologous parasite. Absence of *parasitemia* in BALB/c mice reinfected three and five months after primary parasite clearance indicated the presence of solid protective immunity in these mice. Although comparable levels of $CD19^+$ mature B cells were observed in infection cleared, post-challenged and age matched control mice after 5 months of parasite clearance, frequencies of IgG1 isotype switched memory B cells and $CD19^+CD138^+$ plasmablasts were significantly higher in post challenged mice as compared to infection cleared and age matched, malaria naive controls ($P<0.0001$) suggesting a protective role of memory B cells and plasma cells. Levels of $CD8^+CD44^{hi}CD62L^{low}$ memory cells were significantly higher in immune mice as compared to malaria naïve controls which were not further boosted on challenge. Immune profiling of spleen cell phenotypes indicated that $\alpha\beta$ T cells, $CD4^+$ T cells, $\gamma\delta$ T cells and $Ly6G^+$ neutrophils were significantly higher in post challenged mice as compared to infection cleared mice and age matched controls suggesting a possible role of these immune cell subsets in mediating resistance. Furthermore, frequency of splenic ($CD69^+$) $CD4^+$ and $CD8^+$ activated T cells was significantly higher in post-challenged mice as compared to infection cleared mice and age matched controls indicating that reinfection of BALB/c mice with malaria parasites induced significant T cell activation in spleen. Lastly, the percentage of ($IL10^+$) $CD4^+$ T cells was significantly higher in infection cleared and post challenged mice indicating the existence of anti-inflammatory immune response in these mice. The details of these results and relevance in clinical immunity in endemic areas will be discussed.

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PROTEIN MICROARRAYS REVEAL DIFFERENTIAL ANTIBODY REACTIVITY TO THE *PLASMODIUM FALCIPARUM* PROTEOME IN CHILDREN WITH SYMPTOMATIC MALARIA IN WESTERN KENYA

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Naturally acquired immunity (NAI) to *Plasmodium falciparum* (Pf) is characterized by age-related control of *parasitemia* and protection from

clinical malaria. With the goal of advancing knowledge of how the magnitude and breadth of anti-Pf IgG antibodies (Ab) contribute to NAI, we used plasma from 100 adults (≥18 years) and 100 children (1-14 years) who participated in a treatment time to infection study in western Kenya to probe Pf protein microarrays that represent ~23% of the Pf proteome. (Antigen Discovery, Inc., Irvine, CA) Heat maps of arrays probed with adult and child Ab obtained before anti-malarial cure of *parasitemia* showed that there was significant overlap in the hierarchy of proteins recognized by Ab from both age groups. Adult Ab responses were stronger and reacted with a greater breadth of proteins than those of children. Kaplan-Meier survival analysis for time to infection over 11 weeks of observation showed no correlation with the strength or breadth of Ab responses. In contrast, the strength and breadth of Ab responses were weaker and narrower among children with symptomatic malaria compared with asymptomatic children and adults. Principal component analysis showed clustering of Ab responses to malaria protein subsets among symptomatic children relative to asymptomatic children and adults. Those antigens with significantly greater reactivity in protected than unprotected children (p-values ≤0.01 with Benjamini-Hochberg correction for false positives) included MSP10, MSP1, MSP2, LSA3, several PfEMP1's, ring exported protein-1 and sporozoite threonine and asparagine rich-protein. We conclude that Pf protein microarrays offer insight into targets of NAI and identify potential candidates for inclusion in multi-antigen malaria vaccines.

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CHANGES IN THE LEVELS AND AVIDITIES OF ANTI-MALARIAL ANTIBODIES IN MALIAN CHILDREN OVER THREE TRANSMISSION SEASONS

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There is a general consensus that high levels and broad specificities of antibodies to blood-stage proteins of malaria parasites are essential in reducing the susceptibility to clinical malaria. However, sero-epidemiologic studies to characterize the quantitative and qualitative changes in antibody responses and establish associations with clinical malaria incidence have yielded conflicting results. To address these issues, we took advantage of a well-characterized 3-year longitudinal sero-epidemiological cohort to 1) evaluate the dynamics of seasonal changes in antibody levels and avidities, and 2) assess whether such changes are associated with reduced risk of clinical malaria. The cohort included 3- to 11-year old Malian children (n=240) living permanently in a region where malaria is endemic and seasonal. Plasma samples were collected from the same child before and after each transmission season and all clinical malaria episodes were documented. The levels of IgG specific for four merozoite antigens (AMA1, MSP1, MSP2 and EBA-175) were measured by a standardized ELISA and antibody avidities measured by Surface Plasmon Resonance. While antigen-specific IgG levels generally increased during wet seasons and then decreased during subsequent dry seasons, the magnitude of change in IgG levels among individuals fluctuated from year to year depending on the antigen tested. Children with high IgG titers exhibited greater changes in IgG levels than those with low IgG titers. We observed some seasonal fluctuations in avidities of antigen-specific antibodies among children but observed no cumulative increases in these avidities over the course of the entire three consecutive transmission seasons. There was no association between the magnitude of changes in IgG levels or avidities and reduced risk of malaria. Nonetheless, our findings underscore the need for conducting multi-season longitudinal studies and evaluating multiple antigens in order to identify malaria-protective antibody responses.

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IL-15 MEDIATED SURVIVAL OF INTRAHEPATIC CD8 CENTRAL MEMORY CELLS IS ASSOCIATED WITH LONG-LASTING PROTECTION AGAINST MALARIA INFECTION

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Ag-specific memory T cell responses elicited by infections or vaccinations are inextricably linked to long-lasting protective immunity. Studies of protective immunity amongst residents of malaria endemic areas indicate that memory responses to *Plasmodia* antigens are not adequately developed or maintained. In contrast, multiple exposures to radiation-attenuated Plasmodia sporozoites (γ-spz) induce long-lasting protective immunity to experimental sporozoite challenge. We have previously reported that multiple exposures to *P. berghei* γ-spz (Pb γ-spz) confers protection in B6 mice. Protection in this model is associated with the accumulation of intrahepatic CD8 T cells comprising two major subsets ($T_{E/EM}$ and T_{CM}); while CD8 $T_{E/EM}$ cells are the primary producers of IFN-γ, CD8 T_{CM} cell display high expression of CD122 (IL-15Rβ). IL-15 is well known for its role in influencing the composition of the memory CD8 compartment through regulation of homeostatic proliferation, survival and differentiation into effector populations. To study the essentiality of IL-15 and the role of CD8 T_{CM} cells in protection, we immunized IL-15 KO mice with Pb γ-spz and discovered that, in spite of having reduced numbers of CD8 T cells, these mice are able to respond normally to *Plasmodium* antigens by expanding CD8 $T_{E/EM}$ cells and are protected short-term against a primary challenge with wild-type sporozoites. However, protection is short-lived, owing to reduced expression of Bcl-2 and increased apoptosis of proliferating intrahepatic CD8 T_{CM} in the absence of IL-15. Therefore, we hypothesize that the maintenance of long-lasting protection induced by Pb γ-spz depends on a process whereby intrahepatic CD8 T_{CM} cells, maintained by IL-15-mediated survival and basal proliferation, are conscripted into CD8 $T_{E/EM}$ cell pool during subsequent infections.

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IDENTIFYING KEY TARGETS OF ANTIBODIES TO *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES USING GENETICALLY-MODIFIED PARASITES WITH DISRUPTED SURFACE ANTIGEN EXPRESSION

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Effective clinical immunity that protects against symptomatic malaria in humans develops gradually after repeated exposure to *Plasmodium falciparum*. During intra-erythrocytic development, *Plasmodium falciparum* expresses novel antigens on the surface of infected erythrocytes, including PfEMP1, RIFIN, STEVOR, and SURFIN. Antibodies to surface antigens are typically variant-specific and appear to play an important role in contributing to protective immunity in humans. However, the significance of different surface antigens as targets of acquired immunity remains unclear. In our study, we used an innovative approach to evaluate the importance of surface antigens as antibody targets. This was achieved using genetically-modified *P. falciparum* lines with disrupted parasite protein trafficking, achieved by deletion of the skeletal-binding protein 1 (SBP1-knockout), and *P. falciparum* lines with inhibited expression of PfEMP1 (PfEMP1-knockdown). Currently, only PfEMP1 is known to be trafficked via the SBP1-pathway to the infected erythrocyte surface. We used high-throughput flow cytometry-based assays to measure antibody reactivity to the infected erythrocyte surface and opsonic phagocytosis

assays using samples from cohort studies in Papua New Guinea and Kenya, including children, adults, and pregnant women. Comparison between the parental and genetically-modified parasites allowed us to quantify the proportion of antibodies targeting specific antigens on the infected erythrocyte surface. We found very little antibody response to SBP1-knockout parasites and markedly reduced antibody response to PfEMP1-knockdown parasites. Our results from studies using multiple parasite lines in two geographically different populations suggest that the major surface antigens targeted by human antibodies are dependent on SBP1 for trafficking to the infected erythrocyte surface and are consistent with PfEMP1 being the dominant target of acquired antibodies. These findings enhance our understanding of acquired immunity to human malaria and have significant implications for vaccine development.

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CELLULAR IMMUNE RESPONSES TO A NOVEL MALARIA VACCINE CANDIDATE, PF SCHIZONT EGRESS ANTIGEN-1, IN YOUNG CHILDREN AND ADULTS

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We discovered Pf Schizont Egress Antigen-1 (PfSEA-1) using a differential screening approach with plasma from children who were resistant or susceptible to *falciparum* malaria. Antibodies to the immunorelevant region of PfSEA-1 (rPfSEA-1A, aa 810-1083) predict resistance to severe disease in two yr old children, block schizont egress from infected RBC *in vitro,* and vaccination with rPbSEA-1A protects mice from *P. berghei* ANKA challenge. To advance PfSEA-1 as a vaccine candidate, we have evaluated cellular immune responses to rPfSEA-1A using cryopreserved PBMCs collected from 3 yr old children and adults living in a holoendemic region of western Kenya. In *in vitro* stimulation assays, endotoxin free rPfSEA-1A induced up-regulation of pro-inflammatory and TH1 cytokines in both Kenyan children (n=19) and adults (n=5). rPfSEA-1A stimulated PBMCs from adults produced 1.76-30 fold higher levels of IFN-γ, IL-2, IL-6, IL-8, IL-12, and TNF-α compared to stimulated PBMCs from children (all $P<0.03$). We analyzed the T-cell effector/memory subsets producing these cytokines using multi-parameter flow cytometry. The frequency of $CD4^+$ T cells making IFN-γ in response to rPFSEA-1A stimulation was 2.77 fold higher in adults compared to children and was largely produced by T-central memory ($CD45RA^{hi}$, $CCR7^{hi}$) and T-effector/memory ($CD45^{low}$, $CCR7^{hi}$) in adults but by T- naïve cells in children ($CD45RA^{hi}$, $CCR7^{hi}$). These data confirm that rPfSEA-1A contains broadly reactive T cell epitopes, rPfSEA-1A specific T-cell responses are detectable in young children, and the frequency of these responses increases with age due to natural exposure. We plan to relate rPfSEA-1A cellular responses and resistance to *falciparum* infection in a larger longitudinal study of Kenyan children.

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PLASMODIUM VIVAX DUFFY-BINDING PROTEIN-SPECIFIC MEMORY B CELL FREQUENCIES CORRELATE WITH FUNCTIONAL ANTIBODIES TO PVDBPII AND PERSIST IN AREAS OF LOW MALARIA TRANSMISSION

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Plasmodium vivax (Pv) Duffy-binding protein (PvDBP) engages the reticulocyte surface and is critical for red cell invasion. Naturally-acquired, binding-inhibitory antibodies (BIAbs) to PvDBP correlated with reduced risk of Pv infection in Papua New Guinea. Serum samples from a western Cambodian study of acute Pv malaria episodes revealed high IgG responses to PvDBP antigens that were both strain-transcending and strain-specific. Specificity can change with one amino acid substitution in PvDBP. The basis of persistent PvDBP-specific memory B cells (MBCs) and their relationship to BIAbs, PvDBP-variant specificity, and total antibodies to PvDBP ($PvDBPAb_{tot}$) have not been examined. Sera were screened for BIAbs and $PvDBPAb_{tot}$ *in vitro* by bioplex/inhibition ELISA [N=189, median (range) age = 23 (2-68) years]. All samples had detectable levels of $PvDBPAb_{tot}$ and 4.7% of them had 'high' BIAbs (>80% binding inhibition relative to malaria-naïve donors). At various times [median (range) = 33 (6-45) months] after their acute Pv malaria episode, sera and PBMCs were collected from 20 adults [median (range) age = 28 (20-54) years] with high (N=9), moderate (N=1), and low (N=10) BIAbs. Four of 9 (44%) individuals retained high BIAbs and one individual without previously-detectable BIAbs acquired high BIAbs. $PvDBPAb_{tot}$ levels dropped by 2 to14-fold in 65% of individuals. By ELISPOT, the median (range) frequency of PvDBP-specific MBCs/10^6 PBMCs was 112 (17-976) and comparable to frequencies of MBCs specific for $PvMSP1_{19}$ (median=126) and tetanus toxoid (media=176). The frequency of PvDBP-specific MBCs correlated with BIAb ($r^2=0.48$, $p=0.0007$) but not with $PvDBPAb_{tot}$ levels. The data suggest that a Pv malaria episode induces PvDBP-specific MBC levels that correlate with BIAb levels and persist for months in areas of low Pv endemicity. Thus, PvDBP-specific MBC frequencies may represent a biomarker of immunologic memory to Pv malaria. This study also indicates that functional Abs can wane with low Pv exposure, which may account for the slow acquisition of immunity in some individuals.

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SEROPREVALENCE TO MALARIA PARASITES IN TAK PROVINCE, THAILAND REVEALS MORE FREQUENT EXPOSURE TO *PLASMODIUM SP.* THAN ESTIMATED BY EPIDEMIOLOGICAL SURVEYS

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Malaria is the most important vector-borne disease in Southeast Asia. In Thailand, malaria incidence has been in decline, with the annual parasite incidence dropping to 0.56 in 2007. The Myanmar-Thai border province of Tak is considered mesoendemic for malaria, and both *Plasmodium vivax* (Pv) and *P. falciparum* (Pf) are equally present. As part of the International Centers for Excellence in Malaria Research (ICEMR) - Southeast Asia project, malaria surveillance is conducted in Tak on both the healthy population and hospital patients, and parasite prevalence is reportedly between 1-2%. However, still little is known about the immuno-epidemiology associated with Pf and Pv infections in the region regarding the breadth and targets of the antibody response to the malaria parasites. Our hypothesis is that the serological profiles of the population will reflect the low parasite prevalence in Tak, showing little antibody reactivity to Pv and Pf. To examine this question, we developed a protein microarray displaying the top 500 most immunogenic antigens of these two *Plasmodium* species. Because malaria prevalence is low, we collected whole blood samples from febrile suspected malaria patients to increase the chances of detecting antibody responses. The sera was probed on the microarray and compared to healthy blood donors from the U.S.; genomic DNA was extracted from RBC pellets and screened by PCR for infection confirmation and species-identification. We detected 14 Pv+ (23%), 6 Pf+ (10%), 2 mixed infections (4%) and 38 (63%) PCR-negative samples. Seventy percent (42 of 60) of serum samples were highly reactive to both Pv and Pf antigens, surprisingly including 22 of 38 (57%) PCR-negative samples. Serum from individuals with Pf+ PCR always produced seropositive profiles on the array, whereas serum from Pv+ individuals produced both seronegative (35%) and seropositive (65%) profiles.

Despite the low detectable Pv and Pf infection prevalence in Tak province, there is unexpected substantial antibody reactivity to the malaria parasites, even amongst non-infected individuals.

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POLYMORPHISMS IN CO-STIMULATORY GENES DO NOT AFFECT *PLASMODIUM VIVAX* PARASITE DENSITY

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Plasmodium vivax is the most prevalent malaria species in Brazil, representing more than 80% of clinical cases reported annually from the Amazon region. A growing body of evidence indicates that the immunity is important in the outcome of *P. vivax* infection. Co-stimulation is an important secondary signal that governs the extent, strength and direction of the immune response that follows. Since parasite density has been recognized as important factor in the outcome in malaria infections, we investigated whether polymorphisms in co-stimulatory genes are associated with *P. vivax parasitemia* in malaria patients from Brazilian Amazon Region. The sample included 147 patients infected with *P. vivax* from Goianésia do Pará, a municipality situated on the southwest of Pará state, Brazil. Nine SNPs were analyzed by PCR-RFLP in seven co-stimulatory genes (BAFF, CD28, CTLA4, CD40, CD40L, CD86 and ICOS). Parasitemia was determined by counting the number of parasites in 100 separate fields under oil immersion microscope and converted to the number of parasites per microliter of blood assuming 8,000 leukocytes/µL. Association between the genotypes and parasite density was determined by Mann-Whitney test, with level of significance of 0.05, using R statistical software. All SNPs tested were in Hardy-Weinberg equilibrium. A trend was noted between the allele C of SNP rs_3116496 in CD28 gene and lower parasite density, but these trend was not significant ($p = 0.1$). No significant association was found between the polymorphisms tested and *P. vivax* parasite density. Our results show that the studied polymorphisms do not affect the *P. vivax* parasite density. However, due to the obvious importance of co-stimulatory pathways in malaria, further studies that elucidate the complex host-parasite interactions could be useful for future vaccine development.

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MERCURY LEVELS IN HAIR OF WOMEN IN A NATIVE COMMUNITY, MADRE DE DIOS, PERU: AN ORIGINAL RESEARCH

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Mercury originates neuropsychological disturbances in neonates exposed intrauterine. In addition, as a result of the emergence of illegal gold mining, there has been an increasing level of pollution in the River Madre de Dios, Peru. Not knowing statistically significant data on pollution in women of childbearing age of native communities, we are determining the levels of mercury present in the food chains in women of childbearing age in the native community Ese'eja Palma Real. An observational, descriptive, cross-sectional study. Samples were collected from the hair of women in fertile age (11- 44 years), which were analyzed by atomic absorption test cool mist in the center of toxicological SAC. Sociodemographic information was collected by a questionary. The analysis was performed using the statistical package STATA 11.0 (STATA Corp®, Texas, US),likewise frequencies were used, measures of central tendency and dispersion for qualitative variables. An important percentage of the population 33.33% (n = 20) of the women showed figures of mercury in hair higher than 2 mg/g. Levels were found above the permissible limit of mercury in hair in women childbearing of the native community Ese'eja Palma Real, Madre de Dios, Peru.

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COMPARISON OF TWO COMMUNITIES AFFECTED BY CHOLERA IN KASESE DISTRICT IN UGANDA

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Some sub-counties in Kasese District, experienced frequent cholera outbreaks since the year 2000 to-date, while others did not. The reasons for this difference were not entirely clear nor had they been explored. This study was therefore carried out to try to establish factors why cholera outbreaks were frequent in some areas and not others of the same district. The specific objectives were to study the socio-demographic profiles of the residents of two sub-counties, assess their socio-cultural practices, their environmental sanitation situations as well as the water sources used, cholera carrier status and the antibiotic sensitivity of the cholera organisms responsible for the cholera outbreaks. A cross-sectional study comparing the situations of residents of Karambi and Bugoye was carried out. Focus group discussions, Interviews and observations of the homesteads, latrines and markets for sanitation and hygiene were conducted; Water samples from sources and households were analysed for faecal contamination. Similarly stool samples obtained from victims who had recovered from cholera were cultured and isolated cholera organisms, tested for sensitivity to first line antibiotics. The main findings why cholera remains problematic in Karambi included: poor hygienic practices, (difference significant $p \leq 0.004$),as well as eating communally from the same dish (difference significant $p \leq 0.008$) and unsafe water sources, contaminated with faeces (both *E. coli* and cholera organisms were isolated from R. Mbabaine of Karambi). In Karambi, 36% of the cholera victims who had recovered were found to be asymptomatic carriers of cholera organisms, resistant to first line antibiotics. Factors therefore responsible for the difference in cholera outbreaks in the two communities were: Unsafe water sources, poor hygienic practices; and high carrier status among the Karambi community with cholera organisms resistant to first line antibiotics.

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CHOLERA FROM THE LENS OF HOUSEHOLD HEADS: PERCEIVED SUSCEPTIBILITY AND HYGIENE PRACTICES IN CHOLERA-INFECTED AND NON-INFECTED COMMUNITIES IN IBADAN NORTH WEST LOCAL GOVERNMENT AREA, NIGERIA

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Cholera is a re-emerging disease of public health challenge where water supplies and sanitary conditions still constitute problems. This study therefore designed to determine household heads perceived susceptibility to cholera and hygiene practices in Cholera Infected Communities (CIC) and Non-Infected Communities (NIC) in Ibadan North West Local Government Area, Nigeria. This is a descriptive comparative study. A four-stage sampling technique involving purposive sampling was used in selecting heads of households used for this study. A total of 800 respondents (400 each) were used for this study in both CIC and NIC in IBNW. Interviewer-administered questionnaire and Focus Group Discussion (FGD) were used for data collection. A 7-point scale was used to measured respondents' perceived susceptibility to cholera. Descriptive statistics were used for quantitative data while FGD were subjected to thematic analysis. Mean age of household heads were 48.5±11.8 and 47.0±11.5 years in CIC and NIC respectively. Some (26.7%) and 14.0% of the respondents in CIC and NIC respectively were of the believed that they are at risk of getting cholera. FGD participants also perceived themselves high to cholera due to poor state of their environment. Majority (93.1%) and

91.9% in CIC and NIC respectively were of the perception that cholera can be gotten from spoilt food. Many (56.2%) and 46.8% were of the opinion that ORS can cure any infected person in CIC and NIC respectively. Only (49.5%) and 46.5% of respondents in CIC and NIC respectively usually treat their water before drinking. Few (4.7%) and 11.3% of respondents in CIC and NIC respectively usually use water guard in treating their water. Only (0.9%) and 0.0% of respondents in CIC and NIC respectively are using dispenser for storing water. Respondents' perceived themselves susceptible to cholera infection yet inadequate preventive practices exist. Community health education on proper hygiene should be intensified in the respondents' communities.

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RELATIONSHIP OF FOOD HANDLERS' KNOWLEDGE AND BEHAVIOR TO *ESCHERICHIA COLI* CONTAMINATION ON FOOD SERVING IN CAFETERIA A CAMPUS

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The previous studies showed that foods serving in cafeterias around the campus contaminated by *Escherichia coli.* This research was to know the relationship between the knowledge and behavior of food handlers serving foods in campus and the contamination of *E. coli.* The cross sectional design used to interview 173 food handlers of all cafeterias and foods serving were as samples. Most Probable Number (MPN) used in analyzing *E. coli* in foods conducted in the Laboratory of Environmental Health, Faculty of Public Health, Universitas Indonesia. Chi square test and logistic regression tests used to analyze the data collected. The result showed that a total of 59.54 foods contaminated by *E. coli.* Poor knowledge of food handler in serving food was statistically significant relate with *E. coli.* contamination (p= 0.008; OR= 2.42; CI 95%: 1.30-4.51) and behavior in washing hands had relation with *E. coli.* contamination as well. (p= 0.022; OR= 0.106; CI 95%: 0.01-0.83). Logistic regression found that poor knowledge in serving foods and behavior washing hands before preparing foods were factors contribute in *E. coli.* contamination in foods with OR = 2.70 (CI 95%: 1.42-5.11; p= 0.002) and OR= 0.08; CI 95%: 0.01-0.65; p=0.018) respectively. Poor knowledge of food handler in serving foods was a risk factor, whereas washing hands before serving foods was a protective factor. Food handlers should educate and give training to raise their knowledge and have a proper knowledge and practice in food serving and preparation.

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SHARED SANITATION AND DIARRHEAL DISEASE: EVIDENCE FROM THE DEMOGRAPHIC AND HEALTH SURVEYS AND RURAL ECUADOR

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The WHO/UNICEF Joint Monitoring Program (JMP) defines all shared toilet facilities as unimproved, regardless of the level of technology. The JMP's sanitation ladder is a 4-tier categorization scheme and differentiates between improved facilities and facilities that are shared but otherwise improved. However, there is little evidence to corroborate this policy and even less understanding of the underlying mechanisms that could lead to elevated risk of diarrhea. Using data from 51 Demographic and Health Surveys completed since 2001, we assess whether the prevalence of childhood diarrhea is higher among those with shared facilities. We also compare the prevalence of diarrhea across levels of the JMP sanitation ladder, as well as by the magnitude of sharing (the number of households that share the facility). In the majority of countries, the prevalence of diarrhea was higher among those with shared facilities than among those with a facility that is not shared. The crude prevalence ratio pooled across all 51 countries was 1.10 (95%CL: 1.08-1.14), but it was substantially attenuated after adjusting for confounders (PR=1.02, 95%CL: 1.00-1.05). Sharing appears to be a risk factor in many countries, but confounding plays an important role. The effect of sharing varies widely across countries, making global policy related to this issue challenging. We are currently conducting fieldwork in rural Ecuador to better characterize sharing and its effect on the transmission of diarrheal pathogens.

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ARE THERE CHANGES IN DRINKING WATER MANAGEMENT FOR YOUNG CHILDREN DURING A CHOLERA THREAT IN A POOR PERI-URBAN COMMUNITY IN THE DOMINICAN REPUBLIC?

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Water-linked disease outbreaks typically prompt increased promotion of the importance of safe drinking water with anticipation of improved purification practices where needed. However, there are few reports of changes in household level water management practices in response to such outbreaks. This study aimed to determine changes in drinking water purification for young children by caregivers over time in relationship to a threat of cholera. Household level drinking water management strategies for young children (0-5 years of age) were extracted from caregiver reports obtained through repeated interviews over time at appointments within a pre-existing and ongoing child growth monitoring program in a poor peri-urban community in the Dominican Republic. Patterns of drinking water improvement practices (chlorination, boiling, use of bottled water) over time relative to the timeline of a cholera outbreak were determined. Caregivers (mostly mothers) of 204 children provided 806 data points on drinking water practices between Sept 30, 2010 and July 10, 2012. The first cholera case in neighbouring Haiti was reported in Oct 2010, then in the Dominican Republic in Nov 2010, and then in the study community in April 2011. Over the study period, use of bottled water was the most frequent routine practice employed by child caregivers (52.6%), with infrequent reliance on boiling (12.8%) and household level chlorination (5.8%). No consistent changes in the employment of drinking water improvement strategies were identified over the period of time studied. However, there were short-lived increases in chlorine use in Feb-March, 2011 (to 15.6%) and bottled water use in April-May 2011 (to 78.1%) which also represented the peak use for these strategies during the study period. Further investigation is needed to determine child caregivers' perceptions of disease threat, beliefs about the value of drinking water improvement strategies, and the effectiveness or lack thereof of different health education strategies.

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HOUSEHOLD-LEVEL INITIATED DRINKING WATER IMPROVEMENT STRATEGIES AND CHILD DIARRHEA IN A POOR PERI-URBAN COMMUNITY IN THE DOMINICAN REPUBLIC

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While evidence-based household-level strategies for improving drinking water quality are well known, comparatively little is known about the patterns of typically employed strategies in various settings. This knowledge gap is particularly concerning for high-risk populations in high-risk settings such as for young children in poor districts of low- and middle-income countries. Determining typically employed strategies and their relationship with child diarrhea may provide locally relevant data to inform health education. This study aimed to determine the drinking water improvement strategies employed for children (0-5 years of age) in a peri-urban community near Santo Domingo, Dominican Republic (DR) and examine whether strategies were related to child diarrhea. Caregivers attending a growth monitoring program, which enrolls most of the community's children, participated in standardized health interviews at each appointment which collected information on child diarrhea in the preceding 2 weeks and the frequency the child drank different types

of improved drinking water in the preceding 4 weeks. Caregivers of 199 children agreed to release their interview data. Multiple responses per child were adjusted by weighting. Approximately 22% of children had had diarrhea prior to their appointment (compared with a national prevalence value of 14% found in the most recent DR Demographic and Health Survey). "Always" and "sometimes" using bottled water (53 and 22%, respectively) were the most frequently reported practices, followed by boiling (12 and 11%) and chlorination (6 and 22%). The high levels of bottled water use are consistent with other reports on the DR. No reported strategy use was related to child diarrhea. Possible factors, requiring further investigation, which may explain this lack of relationship, include (i) reported practice not reflecting actual practice, (ii) inadvertent contamination of improved drinking water, and/or (iii) some situations whereby water improvement practices were employed in response to child diarrhea.

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MICROBIAL CONTAMINATION ON PRODUCE AND FARM WORKER HANDS THROUGHOUT THE PRODUCTION PROCESS ON FARMS AND PACKING SHEDS IN NORTHERN MEXICO

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Although produce associated outbreaks are a serious public health burden, few studies have characterized contamination routes or points in the production process where contamination has occurred. Thus, we aim to: 1) quantify contamination by fecal indicators on produce and farm workers' hands at different points in the chain of events surrounding the production and post-harvest handling process, and 2) assess the association of microbial levels between matched produce and hand rinse samples. Produce rinses (160 cantaloupe, jalapeño, and tomato samples) and matched farm worker hand rinses (107) were collected from 9 farms in northern Mexico at four points: before and after harvest, at the point of distribution from the field, and at the packing shed. Generic *E. coli*, Enterococci, fecal *coli*forms, and *coli*phage were enumerated. Logistic regression found that the risk of E.*coli* presence on produce was 7 times greater when the matched handrinse had *E. coli*, (OR=7.1, 95% CI=2.3-21.6), and a significant increased risk was also seen with *coli*phage (OR=95.6, 95% CI=8.5-1076.4). Spearman's correlations indicated that the concentrations of *E. coli* (rho=0.9), Enterococci (rho=0.5), and *coli*phage (rho=0.7) were significantly correlated between hands and produce. Chi-square tests revealed that the prevalence of contamination on produce varied significantly across points in the chain for *E. coli* (χ2=21.9, p<0.001), Enterococci (χ2=23.5, p<0.001), and *coli*phage (χ2 =14.5, p=0.002). The prevalence of *E. coli* on produce increased from 28% before harvest to 67% at the packing shed, Enterococci increased from 69% to 90%, and *coli*phage from 38% to 61%. Hand hygiene and the packing shed environment should be targeted for effective interventions to mitigate the risk of microbial hazards on produce.

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THE EFFECT OF CLIMATIC FACTORS ON CHOLERA INCIDENCE IN THE FAR NORTH REGION OF CAMEROON

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Cholera is considered as a model for climate-related infectious diseases. During the past 15 years, 11 outbreaks of cholera with varying intensity and spatiotemporal extend were reported in the Far North Region of Cameroon. These outbreaks occurred mainly during the rainy season, but it is not known how climate variability influences the incidence of cholera in this region. In this study, the variability pattern of cholera events was studied in association to local climate variables in one of the 30 health districts found in the Far North. We used monthly time series of total reported cholera cases, average monthly rainfall, average monthly temperature and average monthly relative humidity from 1996 to 2011 to explore the association between climatic factors and cholera incidence in the Maroua urban health District. A Generalized Additive Modelling (GAM) framework was used to assess the effect of climatic factors on cholera incidence. A stepwise single predictor approach and a multiple predictors approach were used. In the single predictor approach, it was found that the deviance explained by humidity for example was 19.7%; while the deviance explained by temperature was 26.8%. The results of the multiple predictors model without considering interactions give a deviance explained of 75.6% and 95.4% when considering the interaction between the factors. These results demonstrate that the occurrence of cholera is geared by the combination and interaction of rainfall, humidity and temperature which provide appropriate conditions for the development and spread of vibrion choleric.

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TOTAL MERCURY CONCENTRATIONS IN SAMPLES OF MUSCLE OF THE FRESHWATER FISH, *ASTYANAX BIMACULATUS,* AND IN THE SEDIMENTS FOLLOWING THE FINAL COURSE OF CAPIM STREAM, MINAS GERAIS, BRAZIL

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Aquatic ecosystems around the world are increasingly impacted by heavy metal pollutants mercury. This element cannot be destroyed and, once in the food web, it reaches increasingly progressive concentrations known as bioaccumulation. The present investigation aims at assessing the degrees of total mercury accumulation, in samples of muscle of *Astyanax bimaculatus* (n=192) and in the sediments following the final course of Capim Stream, Minas Gerais, Brazil. *In situ* measurements in the fresh water collections allowed us to acquire data on the physical characterization of the rivers, from which we could infer the behavior of mercury in the stretch of the river under study. The determination of mercury was tested by using an atomic absorption spectrometer coupled with a cold steam-generating device. The stretch of Capim Stream was shallow with warm, buffered water, despite of the presence of slight acid/basic (pH=6.3/7.8) values in some sampled sites. The water has also shown to be little oxygenated (2-6mg/L), with increased values of total suspended solids (72-200 mg/L) and electric conductivity (90-743 µS/cm). Such conditions have enabled theadsorption of mercury to the total suspended solids and organic carbon as well the growth of algae in sites of steady flow of stream. Algae and sediments represent the food basis for *A. bimaculatus*.In the fish muscles, the highest values of mercury were quantified in the specimens collected in the water pond (1350ng/g^{-1}p.f.;1540 ng/g^{-1}p.f) in the late dry season, and in the mouth of the river (1070 ng/g^{-1}p.f.) in the late rainy season. The feeding seasonality of the Lambari and sedimentin both seasons, besides algae in summer has allowed us to suggest that these fish are contaminated with mercury during the feeding period. In general terms,the degrees of total mercury in the muscle of the specimens collected showed to be low (150-1070ng/g^{-1}p.f.), which are in accordance with compatible values for human consumption permitted by Brazilian Legislation (500 ng/g).

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PERCEPTION OF CHOLERA OUTBREAK, ATTITUDE TO REPORTING AND INVESTIGATION AMONG COMMUNITY RESIDENTS IN IBADAN NORTH-WEST LOCAL GOVERNMENT AREA, NIGERIA

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Cholera has potential for outbreak and is a major threat to lives if not responded to early. Rapid containment of an outbreak is largely dependent on the attitude and behavior of the community as well as perceived risk. This study was conducted to determine knowledge of cholera and its control practices, perceived vulnerability and severity to cholera and attitude to reporting and investigation among residents of IBNW LGA, Nigeria. Cross-sectional design was employed. Three-stage random sampling technique was used to select 427 consenting household members aged ≥18 years old. Communities in the LGA were stratified into three groups (28 inner core, 15 transitory and 17 peripheral) and a quarter of the communities in each group was randomly selected. Households were visited and eligible members interviewed using a semi-structured questionnaire. Knowledge was scored on a 19-point scale (score of ≤10 rated poor and ≥11good), perceived vulnerability on 15-point (scores of ≤7 rated low and ≥8 high) while perceived severity was scored on 25-point (≤ 12 rated low and ≥ 13 high). A 24-point scale was used to score attitude to reporting of cholera outbreak (score of ≤ 12 rated negative and ≥ 13 positive). Data were analysed using descriptive statistics, Chi-square test, and logistic regression at p≤ =0.05. Respondents mean age was 35±11.4 years and 70.7% were females. Most (95.3%) of the respondents had good knowledge of cholera. About 71.4% knew the cause of cholera while 97.2% and 96.3% knew diarrhea and vomiting as clinical sign respectively. The commonest source of information during an outbreak was the radio (38.6%). Many respondents (62.3%) perceived their vulnerability to cholera to be low while majority (98.1%) perceived severity of cholera infection to be high. Significantly, respondents residing in the inner core communities perceived themselves more vulnerable to cholera outbreak (OR=23.7: 95%CI 9.64-58.31). Most (71.2%) of the respondents had positive attitude to reporting of cholera outbreak. The good knowledge of cholera, perception of its severity as high and positive attitude to reporting of cholera among this study participants offers a good ground to address more specific risks issues aiming at improving hygiene practices and low perceived vulnerability.

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SUSTAINABLE ACCESS TO SAFE WATER IN HONDURAN HOSPITALS

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Centralized distribution systems improve access to water quantity; however, ensuring water quality is difficult in low-resource settings. Point-of-use water treatment is widely promoted to improve water quality, but is difficult to sustain. Decentralized treatment can provide safe water without reliance on point-of-use products and individual water treatment behavior. Membrane filtration systems are being adopted in a growing number of low-income settings. There is a need to understand the long-term sustainability of these systems. Between 2007-09, General Electric Foundation donated membrane-filtration systems to 4 hospitals in Honduras. In 2012-13, the Center for Global Safe Water at Emory University visited these sites to assess the technological, organizational and contextual factors that affect sustainability. At each hospital, 30 water samples were analyzed for *E. coli* and 25 safe water knowledge, attitude and practice surveys were conducted with staff and patients. These same data were collected at two control hospitals to assess the impact of the treatment systems. Water from hospitals with treatment systems had significantly lower *E. coli* concentrations than hospitals without these systems. Staff in hospitals with treatment systems were more likely to believe the water was safe to drink (24% vs 0%, p=0.02); but there was no difference in the percentages of staff who drank the water (24% vs 11%, p=0.60). We developed a metric to evaluate the sustainability of the systems using 4 domains: on-site capacity, accountability, technical feasibility, and institutional engagement. Domains were scored from 0 to 4 (4 being the most sustainable) based on interview responses and laboratory results. A score of 2 was defined as the cutoff for sustainability. Domain scores showed different strengths and weaknesses related to sustainability. Overall, the hospitals scored near the cutoff (from 1.7-2.4). The domain of technical feasibility had the lowest scores. The treatment systems were often bypassed due to pressure and flow issues within the piped network. Hospitals also lacked access to and funds for critical parts. On-site capacity scores were high but could be improved through increased training and communication. Efforts to support the sustainability of institution-based water treatment devices should focus on improving training and communication as well as assessing technical feasibility prior to implementation.

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THE COMPARTMENT BAG TEST AS A WSH HEALTH BEHAVIOR INTERVENTION TOOL IN MWANZA, TANZANIA

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Microbial contamination is not detectable with the naked eye so the link between water and disease is often not strong enough to influence behavior. Thus, a direct test for contamination of household water may be more effective than solely social marketing messages. Since much of water quality is determined by household treatment and storage, it is important to understand how household knowledge influences action. The Compartment Bag Test (CBT), a liquid culture quantal assay for *E. coli*, was evaluated in this study as a WSH health behavior and educational tool. Forty households in the urban and peri-urban areas of Mwanza, Tanzania participated in a semi-quantitative survey regarding drinking water attitudes and practice. On day 1, households were asked questions regarding water source, treatment, and storage practices and analyzed 100ml of the household drinking water with the CBT under supervision. After incubation for 18-24 hours at 37C, test results were reported back on day 2 to the household and a post-survey was conducted regarding attitudes and reaction. Perception of the safety of drinking water seems to be a key indicator of whether water is treated prior to consumption, since about the same percentage of respondents that perceived their water to be unsafe to drink, also treated their water prior to consumption, 57%. A statistical difference was found in the perception of safety before and after use of CBT (p-value=0.02). A logistic regression was run to determine the magnitude and direction of change. The perception of safety decreased 8.33 times, even when household drinking water quality was found to be safe according to WHO drinking water guidelines; the perception of safety decreased 16.67 times, when household drinking water quality was found to be unsafe. All users say they would recommend the use of the CBT and after seeing results, and 86.7% said they would change treatment practice. The CBT is a potentially useful health behavior and education tool that should be explored further in future WSH interventions.

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PREDICTORS OF FECAL EXPOSURE IN URBAN PUBLIC LATRINES: THE ROLE OF CHARACTERISTICS AND CONDITIONS

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The Millennium Development Goals seek for countries to halve, by 2015, the proportion of people who lack sustainable access to an improved sanitation facility. An "improved" facility is defined as one that ensures hygienic separation of users from human excreta. Characteristics such as flooring or type of design are used as indicators that a household facility can meet that criteria. Facilities that are shared by >2 households are universally considered "unimproved", out of concern that more users can results in poorer hygienic conditions. Shared public latrines are frequently the only feasible option for meeting the sanitation needs of millions living in crowded urban low income cities. There is little evidence that shared facilities pose a greater exposure risk to users than private facilities, nor to describe the characteristics that might influence that risk. We hypothesized public latrines with permeable flooring, a lack of adequate septage containment, and heavier use would be more likely to contain visible feces and microbial contamination. We collected data on characteristics (typology, flooring material, presence of hand washing stations, access, crowding, safety, privacy, and management), conditions (visible feces, flies, smell), and microbial contamination (*E. coli*, enteric viruses) from 29 public latrines in 4 neighborhoods with varying population density and wealth in urban Accra, Ghana. Concentrations of *E. coli* in soil in the vicinity of the facilities (n= 16, mean 104/gram) and on surfaces inside the facilities (n=69, mean 103/100 cm2) were 1-2 logs greater than what has been reported in household latrines of comparable technology. We found no difference (P<0.05) in concentrations of *E. coli* and enteric viruses for flush/pour-flush and bucket latrines, permeable and impermeable flooring, and other characteristics, nor for neighborhood. These results suggest that public latrines in urban environments do pose a risk of exposure to feces and infectious viruses, but exposure is highly variable and cannot be predicted using commonly-observed indicators.

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ARGININOLYSIS AND URICOLYSIS IN *AEDES AEGYPTI* MOSQUITOES REGULATE THE METABOLISM OF UREA AND OTHER NITROGEN WASTE PRODUCTS VIA A CROSS-TALK SIGNALING MECHANISM

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We previously demonstrated that blood-fed *Aedes aegypti* are able to fix, assimilate and excrete nitrogen very efficiently by using multiple metabolic pathways. During this metabolic challenge, female mosquitoes excrete several nitrogen waste products. Two metabolic origins have been proposed for the urea production, either from argininolysis or from uricolysis. We have recently shown that the expression level of arginase (AR) in tissues increases when urate oxidase (UO) is silenced by RNAi, and vice-versa, suggesting a cross-talk between the pathways. Since the blood meal digestion is delayed in mosquitoes injected with dsRNA-AR, dsRNA-UO or both (dsRNA-ARUO), we decided to examine the effect of knockdown on ovarian development by monitoring the vitellogenin levels in the ovaries by western blotting. The data indicate that the uptake of the vitellogenin by the ovaries of the AR, UO or ARUO dsRNA-injected females occurs at a lower rate during the first 48 h after feeding. Mosquitoes injected with dsRNA against AR and UO completed digestion and matured their oocytes by 72 h after feeding. The transient delay in both digestion and vitellogenesis led us to hypothesize that the synthesis and/or excretion of nitrogen waste regulate the expression of genes involved in fixation, assimilation and excretion of ammonia in *A. aegypti*. To verify this hypothesis, the expression patterns of the genes encoding glutamine synthetase, glutamate synthase, glutamate dehydrogenase, alanine aminotransferase, pyrroline-5-carboxylate synthase, pyrroline-5-carboxylate reductase and xanthine dehydrogenase were investigated in fat body from dsRNA-injected females after blood feeding. The silencing of AR, UO or ARUO expression led to a large decrease in the mRNA levels for most of the genes studied. These data demonstrate that the metabolism of nitrogen waste in mosquitoes is finely regulated by a complex cross-talk mechanism. By using this molecular mechanism, blood-fed female mosquitoes control the disposal of excess nitrogen without affecting their survival.

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PHYSIOLOGICAL, MORPHOLOGICAL AND HORMONAL VARIATION IN *ANOPHELES GAMBIAE S.L.* MOSQUITOES EXPOSED TO THE STRESSFUL CONDITIONS OF THE DRY SEASON IN BURKINA FASO, WEST AFRICA

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In tropical savannahs of West Africa, mosquitoes have to cope with extended periods of harsh environmental conditions during the long (6-9 months) dry season. However, their survival mechanisms under aridity and drought remain poorly understood. This study explored the degree of physiological, morphological and hormonal changes that are being prompted by a switch between the rainy and dry season conditions in three members of the *Anopheles gambiae s.l.* complex that coexist in Burkina Faso. Insects were reared in climatic chambers reflecting environmental conditions recorded in the field during the rainy and/or the dry season. Their metabolic fingerprinting and proteins expression were analyzed by gas chromatography - mass spectrometry and 2D-DIGE respectively. Ecdysteroid hormones were quantified using an enzyme immunoassay and finally spiracles were observed under scanning electron microscopy (SEM). Our study revealed that older female mosquitoes reared under dry season conditions were characterized by lower concentration of tricarboxylic acid cycle intermediates and isoleucine, suggesting metabolic and reproduction depression in the dry season conditions. Overexpression of proteins involved in muscles' contraction (myosin light chain) and cuticle thickness and rigidity (cuticular proteins) were observed during the dry season in both An. coluzzii and *An. gambiae*. On the other hand An. coluzzii and An. *arabiensis* considerably reduced their spiracles apertures which are surrounded with high number of trichomes in dry season. Ecdysteroid concentration was much higher in males than in females, suggesting a role of these hormones in shaping *An. gambiae* reproductive strategies and population demography. By exploring physiological and morphological correlates of mosquito local adaptation, our work contributes to unraveling the complex mechanisms underlying the enormous adaptive potential hidden within the *An. gambiae* species complex.

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CURING DENGUE VIRUS INFECTION IN ADULT *AEDES AEGYPTI* BY CHEMICAL INHIBITION OF HOST FACTORS

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Dengue virus (DENV) has become an increasingly important arbovirus transmitted mostly by the *Aedes aegypti* mosquito. Despite the public health burden of dengue, no vaccines or registered drugs are currently available. Thus, there is growing interest in targeting mosquito vectors to control the virus. Studies have identified dengue virus host factors (DVHFs) that allow the virus to infect the mosquito as well as the vertebrate host. Inhibition of these DVHFs in mosquitoes may represent an alternative method to reduce DENV transmission. Here we investigated whether known mammalian DVHF inhibitors can also reduce virus titer in the arthropod vector, *Ae. aegypti*. We applied two known chemical compounds (bafilomycin and mycophenolic acid) to mosquitoes using various treatment methods including injection, topical treatment or sugar and blood co-feeding. By injection, we confirmed that bafilomycin and mycophenolic acid inhibited DENV in the midgut at 7 days post infection by 55% and 66% respectively. It is known that bafilomycin binds to the highly conserved vATPase c subunit of V0 and mycophenolic acid binds to inosine-5′-monophosphate dehydrogenase in human cell lines. We will continue to study anti-DENV effects of the compounds in *Ae. aegypti*.

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STRONG SELECTIVE PRESSURE AGAINST A RECOMBINANT SINDBIS VIRUS THAT INDUCES APOPTOSIS IN THE MOSQUITO *AEDES AEGYPTI*

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Our laboratory recently reported that *Aedes aegypti* mosquitoes which were injected with dsRNA corresponding to the anti-apoptotic gene *Aeiap1* exhibited high levels of midgut apoptosis, and this resulted in enhanced replication and midgut dissemination of Sindbis virus (SINV) following an infectious blood meal. Similarly, silencing the initiator caspase *Aedronc* resulted in decreased SINV replication and dissemination. These results could be interpreted as indicating that apoptosis promotes SINV replication and spread. However, the gene silencing approach affects both infected and uninfected cells, and could have secondary effects. As an alternative approach, we have utilized an alphavirus transducing system to construct a recombinant SINV that induces apoptosis. Oral infection of *A. aegypti* with a SINV expressing the pro-apoptotic *Drosophila* gene *reaper* (MRE/Rpr) induced apoptosis in infected midgut cells, while control viruses with similar size non-coding inserts did not. Replication of MRE/Rpr was reduced and delayed compared to control virus at early time points, but the titers of the two viruses were similar by 7 days post infection (dpi). Sequencing of plaque-purified viruses obtained from mosquitoes infected with MRE/Rpr revealed that beginning at 3 dpi, the majority of MRE/Rpr viruses recovered had deletions that eliminated expression of *reaper*. However, all control viruses recovered from infected mosquitoes contained intact control inserts, even up to 7 dpi. Together these results suggest that there was strong selective pressure against viruses expressing Reaper, and indicate that if apoptosis is triggered in infected cells, it can reduce SINV replication in *A. aegypti*.

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A POTENTIAL ROLE FOR EFFECTOR CASPASES CASPS18 AND CASPS19 IN MIDGUT ESCAPE OF SINDBIS VIRUS IN *AEDES AEGYPTI*

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The midgut epithelium is the first target of arboviruses when they invade the arthropod vector. To establish a disseminated infection, arboviruses must cross the midgut basal lamina (BL), an extracellular layer that is secreted by epithelial cells and prevents passive diffusion by viruses. We are using Sindbis virus (SINV) and the mosquito vector *Aedes aegypti* to understand how arboviruses escape from the midgut and establish systemic infections. During baculovirus infection in lepidopteran larvae, midgut infection initiates a cascade of protease activation in which matrix metalloproteases (MMPs) activate effector caspases, leading to cleavage of BL proteins and remodeling of the BL lining tracheal cells associated with the midgut, which allows baculovirus to escape the midgut. We hypothesize that the MMP-caspase-BL remodeling pathway is also used by arboviruses to escape the mosquito midgut. Prime candidates for caspase involvement in midgut escape and BL remodeling are CASPS18 and CASPS19, effector caspase homologs related to *Drosophila* Decay. Although CASPS18 does not have enzymatic activity, it has been shown to act as a decoy caspase that is able to enhance the activity of CASPS19. The levels of CASPS18 and 19 transcripts and proteins in midgut were not altered by SINV infection, but CASPS19 was cleaved and activated in midgut following a blood meal. RNAi-mediated silencing of CASPS18/19 caused a decrease in midgut caspase activity, and also resulted in lower virus titers than control mosquitoes following an infectious blood meal containing SINV. Immunofluorescence using antisera specific for CASPS18 and 19 revealed that CASPS18 and 19 were expressed in tracheal cells associated with midgut. SINV was also found in tracheal cells in SINV-infected midguts, suggesting that SINV may use the tracheal system to establish systemic infection, and that CASPS18 and 19 may facilitate midgut escape of SINV.

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IDENTIFYING BINDING PARTNERS OF AGDSCAM FOR ITS ANTI-*PLASMODIUM* ACTIVITY IN MOSQUITO

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The insect's immune system use limited numbers of germline-encoded pattern recognition receptors to recognize numerous pathogen-associated molecular patterns. Only after recognition, insects can activate defense responses to eliminate invading pathogens. The Down syndrome cell adhesion molecule (Dscam) was first discovered in *Drosophila* as a highly diverse axon guidance molecule. Subsequent studies showed it was also involved in invertebrate innate immunity. *Anopheles gambiae* Dscam (AgDscam) contains 10 Ig domains and 6 fibronectin repeat domains with the potential of generating over 31,000 alternative splice forms with different pathogen-interaction and inhibition specificities, thus increasing the insect's pattern recognition receptor repertoire. Our previous studies have shown that *An. gambiae* up-regulate splice forms of AgDscam upon *Plasmodium berghei* and *P. falciparum* infections that are specific in the defense against these two pathogens. In order to gain a better understanding of AgDscam-mediated anti-*Plasmodium* defense at the mechanistic level, we performed a yeast-two-hybrid screen using both extracellular and intracellular domains of AgDscam as baits. For the extracellular part, we used the sequence containing the first 8 Ig domains of AgDscam, which showed anti-*Plasmodium* activity in transgenic mosquitoes, as bait and got 7 preys. The intracellular domain turned out to be auto-active for the yeast-two-hybrid assay, therefore we had to cut

it into 3 over-lapping pieces and 2 of them are not auto-active. Using these 2 pieces as baits, we have obtained several preys. We are now in the process of validating the interactions of the proteins with AgDscam and testing their potential functions in anti-*Plasmodium* defense using RNAi-mediated gene silencing.

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FUNCTIONAL CONFIRMATION OF THE IMPORTANT ROLE OF THE CYTOCHROME P450, CYP6M7 IN PYRETHROID RESISTANCE IN THE MAJOR MALARIA VECTOR *ANOPHELES FUNESTUS*

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Pyrethroid resistance in *Anopheles funestus*, one of the main malaria vectors, is threatening malaria control in Africa. Elucidation of the resistance mechanisms is crucial to implement suitable resistance management strategies. Although progress have been made recently with the detection of CYP6P9a and CYP6P9b resistant genes, the complete set of genes responsible for pyrethroid resistance is still unknown. Taking advantage of recent transcriptome sequencing in An. *funestus*, we designed a new whole genome microarray chip to thoroughly investigate pyrethroid resistance mechanisms in this species. Our work has revealed that besides CYP6P9a and CYP6P9b, another P450 gene, CYP6M7, is playing an important role in pyrethroids resistance mainly in southern African countries. A transcription analysis using microarrays and qRT-PCR, shows that CYP6M7 is highly up-regulated in southern African, especially in Zambia where the CYP6P9a and CYP6P9b over-expression is much lower than in Malawi and Mozambique. Functional characterization of CYP6M7 with an *in vitro* metabolic assays using heterologous recombinant CYP6M7 enzyme in *Escherichia coli* confirmed that this P450 can metabolize both type I (permethrin and bifenthrin) and type II (deltamethrin and lambda-cyhalothrin) pyrethroids commonly used in malaria vector control. Additionally, using transgenic Drosophila melanogaster expressing CYP6M7 through a GAL4/UAS system we have established the role of CYP6M7 in resistance profiles against different insecticides. Furthermore, the analysis of the genetic polymorphism of CYP6P9a, CYP6P9b and CYP6M7 revealed that these genes are under antagonistic selection forces, with the CYP6M7 under balancing selection while CYP6P9a and CYP6P9b are both under directional selection in the three countries. This study suggests that CYP6M7 may have a broader substrate spectrum while CYP6P9a and CYP6P9b have a limited substrate range focusing mainly on pyrethroid insecticides.

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DIFFERENTIAL INHIBITION OF EFFECTOR CASPASES CASPS7 AND CASPS8 BY INHIBITOR OF APOPTOSIS (IAP1) IN *AEDES AEGYPTI*

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Our laboratory is studying the regulation of apoptosis in *Aedes aegypti*, a vector for several important arboviruses including dengue, yellow fever and Chikungunya, and whether apoptosis can serve as an antiviral defense in mosquitoes during arbovirus infection. Caspases are cysteine proteases that are important in carrying out apoptosis. Six initiator and five effector caspases have been identified in *A. aegypti*, and three of these, including the initiator caspase Dronc and the effector caspases CASPS7 and CASPS8, have been shown to play important roles in apoptosis. Opposing the action of caspases are the negative regulators Inhibitor of Apoptosis 1 (IAP1) and Defense Repressor 1 (Dnr1). Previously obtained RNAi data indicate that IAP1 functions by inhibiting both Dronc and CASPS7, while Dnr1 specifically inhibits CASPS8. However, although IAP1 appears to act specifically through inhibiting CASPS7, binding assays indicated that IAP1 is able to bind to both CASPS7 and 8, albeit more strongly to CASPS7 than CASPS8. Based on these observations, we hypothesized that IAP1 would be a better inhibitor of CASPS7 than CASPS8. To test this, we produced recombinant IAP1, CASPS7 and CASPS8 to use in inhibition assays. We observed that the same concentrations of CASPS7 and CASPS8 had significantly different caspase activities, possibly due to differences in the amount of activated caspase in the two recombinant protein preparations. To normalize the amount of CASPS7 and CASPS8 in our assays, we used active site titration to determine the amount of each active protein. Using same amount of active CASPS7 and CASPS8, we analyzed the ability of IAP1 to inhibit each caspase and found that that IAP1 was 6-fold better at inhibiting CASPS7 than CASPS8. This result corroborates the binding data, and explains why IAP1 acts preferentially through inhibiting CASPS7 during apoptosis.

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TRANSCRIPTOMICS OF DIFFERENTIAL VECTOR COMPETENCE: WEST NILE VIRUS IN TWO *CULEX* POPULATIONS

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Understanding mechanisms that contribute to viral dissemination in mosquito vectors will contribute to our ability to interfere with the transmission of viral pathogens that impact public health. The expression of genes in two *Culex pipiens quinquefasciatus* populations from Florida with known differences in vector competence to West Nile virus (WNV) were compared using high throughput sequencing. Four day old female mosquitoes from two populations of *Cx. pipiens quinquefasciatus* were fed a blood meal containing 6.0 $\log_{10}$ pfu/ml of West Nile virus. Five days following infection female mosquito bodies were collected and immediately frozen for RNA extraction. Extracted total RNA from each population was sent for transcriptome analysis using Illumina high throughput sequencing. Six RNA-seq libraries were generated from two populations of *Cx. quinquefasciatus*. A total of 15,176 transcripts were combined for comparison of expression differences between the two populations and 118 transcripts were differentially expressed ($p<0.05$). The fold change in expression of the differentially expressed genes ranged from -7.5 - 6.13. The more competent population for WNV (Gainesville) over expressed 77 genes and down regulated 44 genes, compared with the less competent population for WNV (Vero Beach). Preliminary GO function analysis showed that the largest proportion of transcripts was included in the catalytic activity and transporter activity groups except for those in the unknown group. Interestingly, the up- regulated gene set contained most of the catalytic activity function and the down- regulated gene set had a notable proportion of transcripts with transporter activity function. Also, binding and signal transducer activity categories showed different proportions in each up- and down-regulated gene set. Immune response category was shown in only the down regulated gene set, although those represent a relatively small portion of the function. The analysis revealed that the salivary gland genes were over expressed, including gene products involved in odorant binding and blood feeding, in the Gainesville population compared to the Vero Beach population. Validation of the RNA-seq data on a random selection of genes will be discussed.

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CHARACTERIZATION OF A NOVEL BINDING PARTNER OF THE ANTI-*PLASMODIUM* IMMUNE FACTOR FBN9

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In *Anopheles gambiae* mosquitoes, the IMD pathway is of particular importance as it modulates the development of the human malaria parasite, *Plasmodium falciparum.* We have previously shown that one of the downstream effectors of this pathway, the pattern recognition receptor FBN9, is an antagonist of both bacterial and *Plasmodium* infection. As part of an ongoing effort to identify the exact mechanism that various immune

factors employ to defend *Anopheles gambiae* against pathogens, we used a yeast two hybrid screen to identify novel FBN9 binding partners. Here we provide further characterization of the interaction between FNB9 and GPROP10, a GPCR with no previously identified immune function. Our results show that GPROP10 may also be an *Anopheles gambiae* effector gene. Additionally, biochemical analysis was performed to further analyze this association and to identify the binding region of FBN9 on GPROP10. Since GPCRs are major components of many signal transduction pathways understanding the physiological significance of this interaction can help to further elucidate the role of FBN9 in the mosquito.

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THE SIGNIFICANCE OF A MOSQUITO HYPER-VARIABLE PATTERN RECOGNITION RECEPTOR, *AGDSCAM*, IN THE MEMORY OF INNATE IMMUNE SYSTEM

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Unlike that of vertebrates the innate immune system of the mosquito appears to lack the adaptive immunity and immunological memory, which relies on the limited numbers of germ line-encoded pattern recognition receptors to generate the specificity towards the pathogen recognition. The studies of the molecular mechanisms that determine the recognition of the pathogens are of the biggest interest. AgDscam, *A. gambiae* down syndrome cell adhesion molecule, which have the potential to generate over 31,000 alternative splice forms with different interaction specificities. We have shown that AgDscam is an essential hypervariable receptor of the *Anopheles gambiae* immune surveillance system, which produces splice form repertoires that are pathogen challenge-specific. Immune priming is a new paradigm in innate immunity. A recent study with the *Anopheles-Plasmodium* system showed that primed mosquitoes previously challenged with parasites developed fewer parasites than control mosquitoes. This result suggests the knowledge gained from immune priming could be used to development new strategies for malaria control. We hypothesized that the alternative splicing of AgDscam plays an important role in immune priming. We started the assay by challenging mosquitoes with 4 different gram positive and gram negative bacteria, and at different time points post infection we collecting hemolymph to assay the expression of AgDscam 101 exons by using CombiMatrix custom microarray. Further detailed studies are undergoing to investigate whether AgDscam are involved in immune priming and memory in the mosquito innate immune system.

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AEDES AEGYPTI LARVAL PHOTORECEPTORS EXHIBIT PATTERNED EXPRESSION OF RHODOPSINS

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The larval stage of *Aedes aegypti*, the vector for dengue and yellow fever, consists of four larval instars. In all four larval instars, the primary visual system is composed of four ocelli, located on the lateral aspect of the head. The adult compound eye begins to develop during the third instar as a row of individual ommatidial units at the anterior side of the ocelli, and a continuous wave of differentiation then moves in the anterior direction during the third and fourth instar stages to ultimately produce the complete adult eye. We investigated the expression of rhodopsins, G-protein coupled receptors (GPCRs) that initiate visual transduction, in these larval eye structures. Antibodies specific to each rhodopsin were created and used to detect the rhodopsins expressed in the larval retinal structures. The analysis showed that rhodopsins Aaop1, Aaop3, and Aaop7 are each expressed in distinct spatial and temporal patterns. Aaop3 is a major rhodopsin of the larval ocelli photoreceptors. It is localized to photoreceptor cell bodies under light conditions and moves to the photoreceptor rhabdomeres in the dark. Given that the rhabdomeres are the site of phototransduction, the relocation of Aaop3 allows the ocellar photoreceptors to increase light sensitivity under low ambient light conditions. Aaop7 is found in several rows of ommatidia on the anterior edge of the developing compound eye, showing that Aaop7 is expressed only in newly differentiated ommatidial units. Aaop1 was detected later and in the cell bodies of ommatidia within the posterior regions of the developing compound eye. These results reveal that expression of Aaop7 is transient in the developing compound eye and that the expression of Aaop1, the major rhodopsin of the adult R1-6 photoreceptor cells, initiates during these larval stages.

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ROLES OF FIBRINOGEN-RELATED PROTEIN 1 ON *PLASMODIUM* INVASION IN *ANOPHELES GAMBIAE*

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Malaria is a world-wide health problem that affects two thirds of world population, and causes more than 300 million clinical cases and over 650,000 deaths per year. However, the molecular mechanisms responsible for recognizing malaria parasites in mosquitoes are not yet well understood. Recently, we determined that fibrinogen-related protein 1 (FREP1) is significantly associated with *Plasmodium falciparum* parasite invasion by using high-throughput whole genome sequencing and direct association studies between non-synonymous single nucleotide polymorphisms (SNPs) and *P. falciparum* infection in wild *Anopheles gambiae* mosquitoes from Kenya. Surprisingly, knockdown FREP1 expression by RNAi greatly reduced *P. berghei* infection prevalence and intensity in mosquitoes, while over-expression of FREP1 genes increased *P. berghei* infection in *An. gambiae* mosquitoes. Protein sequence analysis and motifs prediction suggest that FREP1 has a 22-amino acid signal peptide at its N-terminal and the rest portion of protein is extracellular, which was validated by expressing FREP1 in insect cells. The FREP1 protein was secreted from High Five insect cell line into medium. Oligo-array data showed that FREP1 was highly expressed in mosquito midguts. Therefore, we propose that FREP1 acts as a receptor for *Plasmodium* parasites in mosquitoes. To further test this hypothesis, we incubated FREP1 proteins with *Plasmodium* ookinetes, and the results indicated that FREP1 proteins bound *P. berghei* ookinetes very well, which supports FREP1 as a receptor of *Plasmodium* parasites during malaria invasion in mosquito midguts.

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THE EFFECT OF VIRULENCE FACTORS ON THE PATHOGENICITY OF *STAPHYLOCOCCUS EPIDERMIDIS* IN RATS AND MICE

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Staphylococcus epidermidis is part of the gut microbiota and is the most frequently isolated species of the coagulase negative staphylococci from human stool. However, it is not clear how its presence in the gut affects the cellular structures and functions of this organ. In this study therefore, the pathogenicity of strains of *S. epidermidis* which were isolated from the stool samples of apparently healthy children was investigated in mice and rats. The albino mice (22_30g) and albino rats (100-155g) of both sexes were infected orally and intraperitoneally with graded doses of the bacteria. Acute infection in these animals caused temporary behavioural changes as shown by restlessness and abdominal stretching but did not result in death even at a dosage of 2 x 10^9cfu/ml. Daily administration of the same dose for 14 days resulted in the death of 11 out of 28 (39.3%) mice. Histopathological examination of the affected organs showed congestions, aggregations and multinucleated hepatocytes in the liver, infiltration of the kidney tubule interstitial by chronic inflammatory cells, coagulative necrosis of the kidney, spleen, intestine and stomach cells

as well as marked stroma fibrosis of the spleen. Coagulative necrosis of cells was the most frequently occurring pathological alteration. Lethality and pathological effects reflected the virulence factors expressed by the organism. The results indicate that *S. epidermidis* strains colonising the gut could cause invasive diseases and serious pathological changes in the gastrointestinal tract.

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HOSPITALIZATIONS AND DEATHS DUE TO DIARRHEAL DISEASE IN CHILDREN UNDER FIVE YEARS OF AGE AT FOUR HOSPITALS IN HAITI, 2010-2012

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Acute gastroenteritis is an important worldwide cause of both hospitalization and death in children under 5 years of age, particularly in low-income countries. In Haiti, where the national introduction of a vaccine against rotavirus, a leading cause of pediatric diarrhea morbidity and mortality, is planned for July 2013, the burden of childhood diarrheal disease is not well understood. We conducted a retrospective review of hospital discharge registries from 2010 to 2012 in the pediatric wards of four hospitals_two in Port-au-Prince, and one each in Artibonite and Southeast Departments_that are part of a laboratory-enhanced syndromic surveillance system in Haiti. We recorded the number of all-cause and diarrhea hospitalizations and deaths by age (<2 and 2-5 years of age) and epidemiological week. A diarrhea hospitalization was defined as one due to diarrhea, acute gastroenteritis, dehydration, or intestinal parasitosis. Of 10,621 total hospitalizations in children under 5, 3,582 (34%) were for diarrhea, including 665 (27% of total hospitalizations), 1,117 (33%), and 1,800 (38%) in 2010, 2011, and 2012, respectively. Eighty-nine percent (3,169/3,582) of pediatric diarrhea hospitalizations were in children under 2. Among 540 deaths in children under 5, 62 (11.5%) were due to diarrhea, and 60 of the 62 diarrheal deaths were in children under 2. The case fatality rate among hospitalized diarrhea patients under 5 was 1.7%. There appeared to be two seasonal peaks in diarrhea hospitalization - a taller February-May peak and a smaller peak in October. From 2010 to 2012, diarrhea was a major cause of hospitalization and death in children under 5 in four hospitals in Haiti; the greatest burden was among children less than 2. The annual increase in the proportion of diarrheal patients among all hospitalized patients during this period may be partly explained by the cholera epidemic, which began in October 2010. Continued hospital-based surveillance of pediatric diarrhea hospitalizations and deaths will enable assessment of the impact of rotavirus vaccine introduction in Haiti.

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IMMUNOGENICITY OF AN ORAL CHOLERA VACCINE, SHANCHOL, IN A LARGE FEASIBILITY STUDY IN BANGLADESH

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Bangladesh is an endemic region for cholera as a result prevention and control of this disease is important using immunoprophylactic measures with vaccines. Immunization against cholera is considered a suitable public health tool for preventive measures since it is difficult to provide safe drinking water and proper hygiene in the near future to high risk areas prone to cholera. Therefore, a study was conducted to ascertain the immunogenicity of cholera vaccine, Shanchol within a large feasibility study in Bangladesh which was conducted in a high risk population in Mirpur in urban Dhaka. From a total of over 141,000 participants, a subgroup of 330 people were from the six study wards of Mirpur (2,4,5,6,14 and 16). The objective was to measure the immunogenicity of the cholera vaccine, Shanchol when administered in a large mass immunization program. The subgroup comprised of adults (18-45 yr: n=110), toddlers (2-5 yr: n-110) and younger children (12-23 mo: n=110). The two-dose regimen of the vaccine was administered orally at least 14 days apart, no serious adverse events was elicited or recorded for any participant throughout the study. Vibriocidal antibody responses in adults were 79% to V. cholerae O1 Inaba, 81% to V. cholerae O1 Ogawa. In the toddlers responses were 87% and 90% to O1 Inaba and Ogawa respectively. In the youngest age group it was 76% and 74% to Inaba and Ogawa respectively. The responses in all ages were higher at day 7 and day 21 compared to pre-immune titers (P<0.001). Overall the antibody response was 81% in all age groups to V. cholerae O1 Inaba and Ogawa. Similar immunogenicity profiles were seen in a previous pilot study which was conducted before initiating the evaluation of large scale feasibility study in Bangladesh. The result of this study is very encouraging, showing that the batch of Shanchol vaccine used was safe as well as immunogenic, giving robust antibody responses in all age groups of individuals. The study thus shows that there is benefit in use of the oral cholera vaccine using the existing national immunization system to target all age groups above 1 year of age in the future in cholera endemic countries like Bangladesh living in high risk conditions.

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VIBRIO CHOLERAE OUTBREAK IN BATALA, PUNJAB, INDIA - 2012

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In India, acute gastroenteritis (AGE) is a major public health problem; in Punjab state, 15 cholera outbreaks out of a total of 39 AGE outbreaks were reported in 2012. In October 2012, a death due to AGE was reported at Civil Hospital Batala, followed by a sudden rise in the number of hospitalized AGE cases. We sought to establish the cause and source of infection. A case was defined as a person who had three or more loose stools per day between 25th September to 10th November 2012 from Gandhi Nagar camp and adjoining areas. To ascertain cases, we conducted a house to house survey in the affected area. Water specimens were tested using the method of "Most Probable Number (MPN)" for potability of water; culture of stool samples was performed to identify the causative agent. Epi-info & MS excel were used for analysis. A total of 834 cases and 33 deaths were identified from population of 24,765 (attack rate (AR): 3.4%, case fatality ratio: 4%). The AR was significantly higher among females (n=440) than males (n=394) (3.7% vs. 3.0%, p=0.002) and among those older than 55 years (n=105) vs. under/equal to 55 years (n=729) (4.8% vs. 3.2%, p<0.001). The most affected area was Gandhi-Nagar Camp (AR=6.6%) followed by Guru Nanak Nagar (AR=2.1%) and Murgi-Mohalla (AR=0.60%). *Vibrio Cholerae* O1 Ogawa was identified in 11 out of 35 stool samples; 12 water samples obtained from 23 households demonstrated fecal contamination. Bacterial growth and contamination from sewage were identified in piped drinking water which served these homes.The piped water supply to the affected area was immediately stopped and an alternate source of water supply (tanker) was arranged; reports of new cases declined. In conclusion, sewage-contaminated water was likely the source of infection. Chlorine tablets and ORS packets were distributed with health education in the community. Prompt public health action through identification of the source of contamination and the implementation of control measures stopped the occurrence of new cases thereby limiting the scale of the outbreak.

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EPIDEMIOLOGY OF NOROVIRUS IN PUERTO MALDONADO, PERU

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Norovirus is the leading cause of acute gastroenteritis worldwide. Direct measurement of community incidence of norovirus gastroenteritis is largely limited to developed country settings. In October 2012 we implemented a prospective population-based cohort study of diarrheal disease comprised of 265 households and 1350 persons in Puerto Maldonado, in the Southern Amazon basin of Peru. Households were visited three times per week and stool samples were collected from persons who experienced diarrhea (defined as three or more loose or liquid stools in any 24 hour period). Samples were tested using real time-reverse transcription polymerase chain reaction (real time RT-PCR). From October 15, 2012 to April 2, 2013, 208 cases of diarrhea were identified (33.8 episodes/100 person-years of follow-up). Samples were obtained from 56.3% (117/208) of persons with diarrhea, of which 16.2% (19/117) were positive for norovirus _ 5.1% (6/117) for genotype I and 11.1% (13/117) for genotype II. The median age of norovirus positive individuals was 2.6 years old (IQR 21.6; SD 24.3). Vomiting was also reported in 36.8% (7/19) of those norovirus positive. Three persons (0.2%) required outpatient care and one person (0.1%) required hospitalization. Based on the assumption that individuals who gave a specimen were representative of the etiology of all cases, norovirus incidence was calculated to be 5.4 episodes per 100 person years. Norovirus, predominantly genogroup II, is a common cause of diarrhea in the area of study. Longitudinal data collection from this cohort will allow us to further understand the economic burden, seasonality, and risk factors for intra-household transmission of norovirus in Peru.

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THE INDIAN OCEAN DIPOLE AND CHOLERA INCIDENCE IN BANGLADESH

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It has been reported that El Niño-Southern Oscillation (ENSO) plays a role in the interannual variation of endemic cholera in Bangladesh. The Indian Ocean Dipole (IOD) is also associated with interannual climate variability in the tropical Indian Ocean. We explored the relationship between the IOD and the incidence of cholera in Bangladesh. A generalized linear negative binomial model was used for time-series regression of the number of monthly hospital visits for cholera in Dhaka and Matlab and the Dipole Mode Index (DMI), controlled for ENSO index (NINO3) and seasonal and interannual variations. We also performed a cross wavelet coherency analysis to examine whether the association between the IOD and the incidence of cholera was stationary (i.e., constant through time). From the generalized linear model, the increased number of cholera cases in Dhaka was associated with a higher DMI at a lag of 0-3 months, while it was also associated with lower DMI at a lag of 4-7 months. In Matlab, increased number of cholera cases was associated with a higher DMI at a lag of 0-3 months and with high NINO3 at a lag of 8-11 months. The increased risk of hospital visits for cholera was associated with high SSH and SST in both areas. Cross wavelet coherency analysis revealed that the strength of both the IOD and ENSO associations with cholera hospitalizations changed across time scales during the study period. In Dhaka, 4-year long coherent cycles were observed between cholera and the index of IOD in 1988-1997. In Matlab, the effect of ENSO was more dominant while there was no evidence for an IOD effect on cholera hospitalizations. Our findings support a hypothesis that a negative and positive dipole event may increase potential flooding and thus an outbreak of cholera in Bangladesh in different lag time whilst the association was time-varying.

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CLOSTRIDIUM DIFFICILE: AN EMERGING ZOONOSIS?

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Clostridium difficile is an anaerobic bacillus that can be extremely pathogenic. This infection occurs in both humans and animals such as canines, equines, and bovines. Human infections typically are nosocomial, however, investigators recently have noted an emerging trend of community-acquired cases. Although no confirmed cases of foodborne *C. difficile* have been reported, the pathogen has been found in retail meat products. Moreover, it is a common pathogen in domesticated animals such as canines. This review examines potential risks for community-acquired *C. difficile* and explores further research in this area.

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EVALUATION OF RISK FACTORS AND CARRIAGE OF ENTERIC PATHOGENS IN CHILDHOOD DIARRHEA IN FOUR RURAL COMMUNITIES IN HAINAN, CHINA

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China's Ministry of Health, with UNICEF, WHO, and UNFPA, found disparities in child mortality between urban and rural areas of China. Rural areas accounted for over 70% of childhood deaths of which 18% were from Diarrhea. This study focused on the determinants and characteristics of diarrheal disease in children under five years old in four communities of Hainan, China. A survey was completed by the primary caregiver of 413 randomly selected children and a stool specimen was collected. BioFire Diagnostics, Inc. analyzed 105 stool samples using a Film Array (multiplexed PCR device) GI panel for detection of 23 enteric pathogens. Water samples were collected from each family's water source and analyzed for *coli*form forming units (CFU) and *E. coli*. Survey results showed 23% of children had diarrhea in the previous 2 weeks and BioFire testing identified an average of 3.6 pathogens per child. Fecal specimens were positive for the following 18 pathogens: EAEC 73%, ETEC 71%, EPEC 76%, STEC 19%, *Campylobacter* 15%, *Shigella*/EIEC 16%, *Aeromonas* 4%, *P. shigelloides* 6%, *C. difficile* 4%, *Salmonella* 3%, *V. cholera* 1%, *G. lamblia* 28%, *Cryptosporidium* 9%, *Adenovirus* 6%, *Norovirus* 5%, Human *Astrovirus* 2%, *Sapovirus* 2%, and *Rotavirus* 1%. Water in all communities was contaminated with fecal flora. The average MPN of fecal flora/100ml of water was: unprotected well water 31,067; public tap/standpipe 25,330; protected well 19,906; tubewell/borehole 5,244; piped into dwelling 3,706; bottled water 925; and piped water 732. Decreased diarrhea correlated with soap observed in household, use of soap in past 24 hours, piped water source and utilization of toilet facilities. Education of mother and proximity of wells to animals or toilet facilities did not influence prevalence of diarrhea. The number of enteric pathogens per child was not influenced by the risk factors studied. With multiple pathogens per child it is difficult to determine the etiological cause of diarrhea. Presence of *Cryptosporidium, Shigella, Campylobacter* and *Giardia* was associated with increased incidence of diarrhea. The number of enteric pathogens per child suggests that these children live in a highly contaminated environment and may be vectors of gastrointestinal disease. This data will be discussed with health care providers and study participants to generate strategies to reduce childhood diarrhea.

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MODELING CHOLERA IN A PATCHY ENVIRONMENT WITH WATER AND HUMAN MOVEMENT

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The World Health Organization estimates that there are 3m-5m cholera cases per year with 100,000 deaths across 40-50 countries. For example, there has been a recent cholera outbreak in Haiti. Cholera is a waterborne bacterial disease caused by the bacterium "*Vibrio cholerae*", which is an aquatic organism. The movement of both humans and water have recently been suggested to influence the spatial spread of cholera in Haiti. To better understand this spatial spread, a new compartmental cholera model is formulated that incorporates patch structure, and both water and human movement. The water and human movement connect individual patches (communities), resulting mathematically a weighted directed graph (a community network). When can a disease like cholera invade this network? Our mathematical results show analytically that the answer to this question depends on both the network structure as well as on the properties of the individual patches. In some situations, the basic reproduction number, which determines the invasibility of cholera on the community network, becomes a weighted average of the patch reproduction numbers, with weights given by the network structure in terms of the net inflow. That is, patches with the most net inflow have the greatest impact on invasibility. Our results also show that clustering disease hot spots together with respect to meta-communities increases the ability of the disease to invade.

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FREQUENCY OF SERINE PROTEASE AUTOTRANSPORTER PROTEINS *ENTEROBACTERIACEAE* (SPATE) IN *ESCHERICHIA COLI* DIFFUSELY ADHERENT (DAEC) ISOLATED FROM CHILDREN WITH AND WITHOUT DIARRHEA

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Diffusely adherent *Escherichia coli* (DAEC) strains have been recognized as the sixth class of diarrheagenic *E. coli* (DEC) and appear as a heterogeneous group. Previous studies have shown the frequency of SPATE genes as virulence factors in the DEC however have not been identified in strains DAEC. The aim of this study was to analyze the frequency of 6 SPATE encoding genes in DAEC isolates from children with and without diarrhea. We have analyzed 104 isolates from children with diarrhea (63) and no diarrhea (41). Two conventional multiplex PCR were used to identify the presence of six virulence genes belonging to the SPATE group (*sigA, pet, espP)* and *(sat, pic,espC*). The EAEC O42 (*pet, pic*), EHEC 933 (*espP*), EPEC 2348/64 (*espC*) and *Shigella flexneri* 1106 (*sigA, sat*) were used as positive controls. *sat* gene was identified as the most prevalent in both cases 40% (25/63) and controls 42% (17/41), followed by *espP* with a frequency of 21% (13/63) for cases and 10% (4/41) for controls, *pet* 8% (5/63) for diarrheal cases and 5% (2/41) in controls, *sigA* 8% (5/63) in diarrhea and 12% (5/41) in controls; *espC* and pic 5% (2/41) only in controls. With these results we conclude that DAEC strains may possess SPATE-encoding genes irrespectiveness if they are isolated in patients with diarrhea or healthy children.

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PREVALENCE OF CAPSULAR TYPES OF *CAMPYLOBACTER JEJUNI* ISOLATES FROM SYMPTOMATIC AND ASYMPTOMATIC CHILDREN IN THE PERUVIAN AMAZON

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Campylobacter jejuni is one of the leading causes of diarrhea worldwide and the development of a protective vaccine is critical. Capsular polysaccharides (CPS) conjugate vaccines have been shown to be protective against *C. jejuni* infections in non-human primates. Continued pursuit of such a vaccine strategy will require answering questions about the required valency of a broadly effective CPS conjugate vaccine against *C. jejuni*. Of importance to this effort will be determining the prevalence of CPS types circulating globally. As a step in that direction, a multiplex PCR assay for detection of CPS encoding genes capable of distinguishing 24 of the 47 CPS types was performed on *Campylobacter* isolates from a longitudinal case-control study conducted between 2002 and 2006 in a cohort of children under 72 months of age, located in a rural community in Iquitos, Peru. This study included 131 symptomatic and asymptomatic individuals of which 206 *Campylobacter jejuni* isolates were acquired from stool samples. Multiplex PCR results demonstrated that the most common CPS types were HS15 (17%), HS8/HS17 (14%) and HS3 complex (12%), while 7% of isolates were non-typeable. Differences in CPS type distribution between symptomatic and asymptomatic infections for HS15, HS8/HS17 and HS3 complex were not statistically significant, while HS2 was detected in symptomatic cases only. Most subjects (39 out of 45) had multiple infections of which each were with an isolate of a different CPS type, suggesting that acquired immunity may be protective. This data represents the first report examining the distribution of CPS types in South America.

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PREVALENCE OF BACTERIAL ENTEROPATHOGENS AND THEIR ANTIBIOTIC RESISTANT PROFILES IN STOOL SAMPLES FROM CHILDREN IN THE PERUVIAN AMAZON

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Diarrheal diseases have a substantial impact on childhood development and mortality in developing countries. Our study was conducted to determine the most common enteropathogenic bacteria in children with in the small Amazon town of Santa Clara just outside of Iquitos-Peru. During this study, 203 children under 5 years of age were monitored bi-weekly for 7 months with asymptomatic stool samples taken monthly and diarrheal stool samples taken when sick. Fecal samples from both symptomatic and asymptomatic participants were cultured on standard media with antibiotic susceptibility determined by disk diffusion. A total of 1925 fecal samples were cultured, from which 500 represented diarrheas. Of the samples tested 12.78% (246/1925) were positive for enteropathogenic bacteria, representing 13.8% (69/500) of the diarrheal samples and 12.42% (177/1425) of the non-diarrhea samples (p=0.4). In total, 257 bacteria were isolated (235 samples with a single pathogen and 11 samples with 2 pathogens isolated). *Campylobacter* (63.6% *C. jejuni* and 36.4% *C. coli*) was the most common enteropathogen present in 72.76% of the isolates, followed by *Shigella* (16.34%), *Plesiomonas shigelloides* (8.17%), *Aeromonas hydrophila* (1.17%), *Salmonella* (1.17%), and one isolate of *Vibrio cholera* Non O1 (0.39%). Of the *Campylobacter* isolates, 81.28% were resistant to ciprofloxacin,67.38% to trimethoprim/sulfamethoxazole,

44.39% to ampicillin, 41.18% to tetracycline, and 11.76% to azithromycin. Among *Shigella* isolates, resistance to tetracycline was 97.62%, ampicillin (80.94%), trimethoprim/sulfamethoxazole (78.57%), azithromycin (11.90%) and ciprofloxacin (0.00%). Our study showed that *Campylobacter* was the most common enteropathogen among both symptomatic and asymptomatic children and was found to be resistant to multiple antibiotics. Our data suggests that children can also be asymptomatic carriers of *Campylobacter*, which would be a significant public health problem in developing countries.

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PATTERNS OF BACTERIAL ECOLOGY AND ANTIBIOTIC RESISTANCE IN TWO REGIONS OF PERU, 2007-2009

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Antibiotic use in Peru lacks regulation, therefore the effective treatment of diarrheal disease is often complicated by antibiotic resistant organisms. In this study, we investigated the patterns of antibiotic resistance in coastal and jungle regions of Peru. From 2007-2009, fecal samples were collected from healthy children under 5 years living in the Ica province, located on the coast, and the Alto Amazonas province, located in the Amazon rainforest. Samples were plated on MacConkey agar for the detection of lactose fermentation, TCBS for *Vibrio*, charcoal agar for *Campylobacter*, and Hektoen agar for *Shigella* and *Salmonella*. PCR was performed for *E. coli* typing and bacterial isolates were tested for antibiotic resistance by disk diffusion. Pathogenic bacteria were isolated from 11% (80/744) of the samples cultured. Of 76 samples from which a single pathogen was isolated, the type of bacteria differed between the coast and the jungle ($p<0.001$). Ninety percent (43/48) of single-pathogen isolates from the jungle were identified as enterotoxigenic *E. coli*; of the coastal isolates, 36% (10/28) were identified as *Campylobacter jejuni*, 36% (10/28) as *Aeromonas,* and 0% as enterotoxigenic *E. coli.* Overall, 99% of single-pathogen isolates demonstrated resistance to at least one antibiotic, with 77% demonstrating resistance to ampicillin, 58% to erythromycin, 51% to each of cotrimoxazole and cephalotin, and 49% to tetracycline. Fifty-two percent of all single-pathogen isolates were resistant to more than one antibiotic. Isolates from the jungle displayed greater resistance to erythromycin (81% vs. 21%), cotrimoxazole (63% vs. 8%), and tetracycline (56% vs. 23%; all ps<0.04), while resistance to ciprofloxacin was greater on the coast (55 vs. 6%; $p<0.001$). These findings suggest that some degree of antibiotic resistance is nearly universal among bacteria isolated from the Peruvian jungle and coast, and that regional differences in resistance patterns should be considered in the treatment of enteric disease in Peru.

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HUMAN DIARRHEA INFECTIONS ASSOCIATED WITH DOMESTIC ANIMAL HUSBANDRY: A SYSTEMATIC REVIEW

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The majority of infectious diseases that affect humans have a zoonotic origin. While zoonotic diseases are commonly discussed from the perspective of emerging infectious diseases, certain enteric pathogens that affect humans have animal reservoirs. Available data on the relationship between enteric pathogens in animals and diarrheal illness in humans has not been examined systematically in the context of domestic animal husbandry. Further exploration of the risk that domestic poultry and livestock pose to human health is required to identify hazards and mitigation strategies. We conducted a systematic review, and meta-analysis if possible, to determine the impact of domestic husbandry of poultry and livestock on diarrheal disease in humans. PubMed/Embase/ISI Web of Science were searched until February 25, 2013 without restrictions on language or year or publication. Bibliographies of selected articles were searched. Eighteen studies met our eligibility criteria. All types of studies with data on presence of domestic animals (poultry, ruminants, goats and swine) and diarrhea in humans were considered. Odds ratios reporting association between domestic animal exposure on diarrheal illness were extracted from the literature or calculated using provided data. Quality of each study was assessed using grading criteria set by the authors. All studies were divided among 18 animal exposure-disease strata; only one stratum containing a sufficient number of studies to perform a random effects meta-analysis. Domestic exposure to poultry was significantly associated with human campylobacteriosis (OR 2.49, 95% CI 1.63-3.81). In addition, 14/19 studies included in this review reported a positive association between domestic animal exposure and diarrheal illness. In conclusion, our results indicate domestic poultry and livestock exposure is associated with increased risk of diarrheal illness in humans. There was a potential for biases, both within our study (publication bias) and between our studies (principally recall bias). There was also considerable heterogeneity of effect among the studies included for meta-analysis. Despite these limitations, we found evidence that domestic animal exposure should be considered a risk factor for human diarrheal illness. Further study may confirm and clarify this relationship and interventions, such as corralling and pasturing, should be explored.

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BACTERIAL AND PARASITIC ETIOLOGIES OF DIARRHEAL DISEASE IN THE PERUVIAN AMAZON, 2003-2011

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Diarrheal disease is a major threat to military populations and has a high prevalence in the Amazonian region of Peru. From 2003 to 2011, diarrheal disease surveillance was conducted among personnel at the Vargas-Guerra Army recruit training base in Iquitos, Peru. All asymptomatic individuals newly stationed at the base were invited to participate in providing a baseline stool sample, and all subjects experiencing diarrhea during their time on the base were requested to present to the Army health post where a stool sample was taken for stool culture and ova and protozoan parasitic detection by microscopy. We conducted a case-control analysis of these data to identify the bacterial and parasitic etiologies of diarrhea in this population. Of 644 diarrhea cases who provided stool samples, 522 (81.0%) could be matched by calendar month with appropriate control baseline samples (which were provided after at least 7 days on the base). *Cryptosporidium* (24.5%), *Giardia lamblia* (18.9%), and *E. histolytic* (16.4%) were the most frequently detected pathogens in samples. *Shigella flexneri Enterotoxigenic E. coli* (ETEC) *and Campylobacter sp.* were detected in 10.3%, 7.1% and 2.0% of samples, respectively. Diarrhea case status was associated with the detection of *Shigella flexneri* (Odds Ratio (OR)=5.71, 95% CI=3.39, 9.63), ETEC (OR=3.02, 95% CI=1.76, 5.18), and *Cryptosporidium* (OR=1.57, 95% CI=1.18, 2.09). No other pathogens assessed were significantly associated with the risk of diarrhea. These data demonstrate the diversity of the etiology on diarrheal diseases in a military population in the Amazon and the importance of *Shigella flexneri*, *Enterotoxigenic E. coli* and *Cryptosporidium* as diarrheagenic agents in this population, which highlight the need for measures to prevent infection and transmission of these pathogens in the region.

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EVALUATION OF *CLOSTRIDIUM DIFFICILE* TREATMENT WITH FECAL MICROBIOTA TRANSPLANTATION

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Clostridium difficile is a gram positive spore-forming anaerobic bacterium that is responsible for causing diarrheal disease around the globe. This bacterium is found in increasing prevalence worldwide causing rising rates of infection. *C. difficile* infection (CDI) is reported primarily as a nosocomial disease and is estimated to cost the U.S. over $3.2 billion dollars a year in healthcare. CDIs are becoming more common, serious, difficult to treat, and more likely to recur. The CDC states that CDI claims 14,000 American lives each year. Current treatment for CDI involves high-powered antibiotic therapy. *C. difficile* strains are becoming more resilient and less effected by antibiotic treatment, especially in relapsing cases. This review was completed in order to examine a treatment option that may prove to be beneficial in the fight to cure people with chronic CDIs. The purpose of this review is to examine fecal microbiota transplantation (FMT) as a potential treatment for CDIs. FMT needs to be further studied as a possible future treatment of CDIs. The following key words were used in order to conduct a thorough review of the pertinent literature: *C. difficile* infection (CDI), fecal transplants, and *C. difficile*. Articles were primarily found through PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/). General guidelines for CDI treatment was discussed in the literature and then compared to the treatment with FMT. The methodology and success rates of the various treatments for CDI were also examined. Evidence from multiple case reports and case studies revealed high success rates for treatment of CDI with FMT. Results of the first randomized clinical trial (RCT) also supports better patient outcome with FMT therapy in people with relapsing CDI. In-fact, results using FMT therapy had significantly higher efficacy for the treatment of recurrent CDI than the use of vancomycin; which is the standard antibiotic used to treat CDIs. Conventional antibiotic therapy eliminates the normal flora of the GI tract, making optimal conditions for *C. difficile* to flourish. FMT helps recolonize the GI tract of the natural flora using donor feces. Fecal transplantation reveals a hopeful outlook as a potential future cure for CDIs, as well as a possible treatment option for a various number of bowel maladies. This opens the door for future research to learn of the potential benefits using FMT for a wide array of conditions.

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ESTIMATING THE FORCE OF INFECTION OF ENTERIC PATHOGENS

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Diarrheal disease remains the second highest cause of childhood mortality despite knowledge of, and improvements to, interventions. A better understanding of the mechanisms that result in exposure to and infection from specific enteric pathogens is necessary to better target interventions and measure their impact. A key mechanism is immunity. The incidence of diarrheal episodes is frequently reported to decrease as children age and observational studies have inferred that this phenomenon indicates the acquisition of immunity that reduces the incidence of infection. Additionally, incidence of diarrhea in breast-fed children is lower than in weaned/not breast-fed children of the same age. Here we present an analysis of empirical age-prevalence data from MAL-ED, a longitudinal study of enteric disease in children from 8 populations. For each of a number of common enteric pathogens we apportions the risk of pathogen presence into the probabilities of population-specific exposure to enteric pathogens, the probability of infection as a function of age and the probability of re-infection reflecting previous exposures. We contrast the difference in the quantitative combination of these three components between pathogens and discuss how knowledge of these biological mechanisms informs possible intervention options.

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NEUTROPHILS EXHIBIT JANUS-LIKE BEHAVIOR DURING *ORIENTIA TSUTSUGAMUSHI* INFECTION IN A MURINE MODEL OF SCRUB TYPHUS

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Scrub typhus is a seriously neglected disease with approximately one-third of the world's population at risk of being infected with *Orientia tsutsugamushi*, and the occurrence of over one million scrub typhus cases annually illustrate its importance in global health. *S*crub typhus is caused by a rickettsia transmitted by the parasitic larval stage of trombiculid mites, primarily of the genus *Leptotrombidium*. All scrub typhus case studies that report blood cell counts, describe neutrophilia during the course of infection. Patients with confirmed scrub typhus have significant increases in activated neutrophil proteins in serum, and the increase of neutrophil recruiting cytokines. We also observed neutrophilia in intravenously infected mice, suggesting key role for neutrophils in scrub typhus disease progression. To determine the role of neutrophils in this infection, female C57BL/6 mice were lethally challenged, and neutrophils were depleted one day prior to infection (D-1), one (D+1), six (D+6), or one and six (D+1/6) days post infection. The effects of neutrophil depletion were observed to be dependent on the time post infection. Animals depleted early (D-1 and D+1) or twice post infection exhibited more severe pathology at an earlier time point and had disease progression similar to non-depleted animals but had greater survival and lower bacterial loads in the organs. Animals depleted 6 dpi recovered weight, and signs of illness had resolved by 12 dpi. Histopathology demonstrated decreased cellular infiltrates when compared to infected, non-depleted animals. Depletion of neutrophils decreased mortality independent of when depleted, but only depletion 6 dpi resulted in amelioration of signs and early weight recovery. These data suggest a dual role of neutropils in bacterial clearance and tissue pathology during scrub typhus infection.

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INJECTIONAL ANTHRAX: AN EMERGING PUBLIC HEALTH ISSUE

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Bacillus anthracis is the pathogen that causes anthrax. Historically, clinicians have classified into three types: cutaneous, gastrointestinal, and inhalational. More recently, however, a fourth form, injectional anthrax, has emerged among heroin users in Europe. First identified in 2000 in a single intravenous drug user, additional cases of injectional anthrax soon were reported throughout Europe over the following decade. Transmission occurs when intravenous heroin, surreptitiously contaminated with *B. anthracis*, is injected into a vein of the addict. The purpose of this study is to review the existing literature on injectional anthrax and to assess the significance of its emergence for physicians and public health officials. The following key words were used to conduct an extensive review of the pertinent literature: Bacillus anthracis, anthrax, heroin, injectional, drug use. Once identified, each article was analyzed for descriptors of patients (age, sex, drug use, nationality), signs/symptoms, outcome (death or recovery), and advice to physicians, if present. Due to a lack of identifiers among the patients/cases, it is difficult to determine their exact numbers. As of early 2013, over 130 different suspected cases were reported, the majority of which (n =119) occurred in Scotland. All patients

identified were intravenous drug users who have used heroin within the previous 1-4 days. Symptoms ranged from mild cutaneous lesions to septic shock, with the majority presenting with sever soft tissue infections at the injection site. Although the mortality rate improved through the outbreak as physicians became more aware of the infections and followed more cautious protocol, mortality still remained around 30%. As 90% of the world's heroin originates in Afghanistan, an area where anthrax is endemic, clinicians and public health officials should be increasingly aware of this novel form of anthrax.

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EPIDEMIOLOGY OF Q FEVER IN THAILAND

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Q fever is zoonotic disease caused by the bacterium *Coxiella burnetii*. This bacterium has been identified as a cause of endocarditis in Thailand, but data on animal reservoirs and frequency of human infection are lacking. Three different human and animal populations were screened in an attempt to determine the prevalence *C. burnetii* antibody and identify animal reservoirs of the disease in Thailand. All sera were tested by commercial ELISA kits for the presence of *C. burnetii* IgG antibody. Human ELISA positive sera were confirmed and titrated by Indirect Fluorescent Antibody assay using phase 2 antigen. Samples with IFA titer $\geq$ 64 were considered positive for *C. burnetii* IgG antibody. First, patients hospitalized with prolonged fever of unknown origin at an academic hospital in Bangkok and participating in a rickettsial disease study were included in the investigation. Acute serum samples from 28 of 152 patients (18%) were IgG positive, indicating evidence of *C. burnetti* exposure, while convalescent testing is pending. In the second screening, a single serum samples were collected from dairy cattle, goats and their owners from farming communities in Chiangmai, Nakonratchasima and Nakonsithammarat provinces of Thailand from January 2012 through June 2013. Cattle showed 4% (28/780), and goats 6% (17/300) positivity for *C. burnetii* antibody. Twenty-seven of 56 livestock farmers' sera (48%) were positive for *C. burnetii* IgG antibody. In the third population, captive wildlife and their caretakers in Chiangmai zoos were screened in May 2012. Of 61 captive wild deer and ruminant species, none were positive, while 12 of 104 wildlife caretakers' sera (12%) were positive for *C. burnetii* IgG. These results demonstrated the high prevalence of *C. burnetii* antibodies among persons with prolonged fever and those with animal exposure and identified likely animal reservoir. More extensive investigations are needed to determine disease risk factors and to describe the clinical presentation and incidence of acute human infection.

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RECOMBINASE POLYMERASE AMPLIFICATION ASSAY FOR RAPID DETECTION OF Q FEVER

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Q fever is a worldwide zoonotic infection caused by the intracellular bacterium *Coxiella burnetti*. Transmission generally occurs via inhalation of aerosolized bacteria in air that is contaminated with infected animal tissues. The clinical syndrome of illness caused by *Coxiella burnetti* is often non-specific which can make diagnosis challenging. In the current diagnostic scheme comparison of serologic samples in both the acute and convalescent stages of infection in conjunction with a clinical syndrome that is consistent with Q fever are the diagnostic standards. Polymerase chain reaction techniques have been developed which are rapid, sensitive and qualitative for the detection and diagnosis of acute (< 2 weeks of symptoms) Q fever. To date there have been no assays developed for rapid and accurate point of care or field testing for Q fever. Recombinase polymerase amplification (RPA) is a novel technology for the amplification and detection of DNA or RNA. RPA is an isothermal amplification process that uses the combined properties of the bacterial recominase (RecA) polymerase and exonuclease**,** to achieve the amplification of specific DNA sequences. The advantage of RPA is that amplification can be done rapidly (< 20 minutes) without the need for expensive thermocycling equipment. Product can be detected with the use of a simple sandwich assay which also eliminates the need for additional detection instruments. The goal of this project was to adapt the RPA assay for detection of Q fever targeting the *IS1111a* transposase gene which is present in multiple copies in the *Coxiella burnetti* genome. Several sets of unique primer sequences were designed for the *IS1111a* transposase gene and the reactions were carried out at 37°C for 20 minutes. The performance of many different combinations of forward and reversed primers was evaluated. The sequences of the probes were designed for the two best primer sets. Endpoint detection of amplicon has been achieved using the TwistAmp nfo kit (*TwistDx*) in conjunction with the Type II Best Cassette (*Biohelix*). This assay showed a detection limit of 25 copies per reaction. To our knowledge our assay is the first developed using RPA for the molecular detection of Q fever. The speed and portability of this assay could be useful in both point of care and field diagnostics for acute Q fever infection.

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CHARACTERIZATION OF CLASS 1 INTEGRONS IN *ESCHERICHIA COLI* ISOLATES FROM BLOOD ORIGIN IN PERUVIAN CHILDREN

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Escherichia coli is the species that most frequently causes gram-negative *bacteremia*. Resistance of gram-negative organisms to antibiotics such as β-lactams, aminoglycosides, trimethoprim and chloramphenicol is caused by many different acquired genes, and a substantial proportion of these are part of small mobile elements known as gene cassettes. Gene cassettes can move into or out of a specific site *(attI* site) in a companion element called as integron, and integration or excision of the cassettes is catalyzed by a site-specific recombinase (IntI) that is encoded by the integron. Integrons are genetic structures able to capture, excise and express genes, frequently included in mobile elements such as plasmids that allow their dissemination among bacteria. This study presents the antibiotic susceptibility and the prevalence and characterization of Class 1 integrons in clinical *E. coli* isolates from blood. Antibiotic resistance was analyzed by the method of Kirby Bauer and the presence of class 1 integrons was determined by PCR in 64 *E. coli* causing *bacteremia* in hospitalized children younger than 5 year of age. To determine the composition of variable regions, amplified products were digested with *HinfI,* and resolved in 2% agarose gels. Representative samples from each RFLP pattern were purified and sequenced. *E. coli* from blood exhibited high levels of antimicrobial drug resistance in blood ampicillin (91%), cotrimoxazole (67%), tetracycline (52%), and gentamicin (44%). Integrons type 1 were found in 22 (31%) isolates, in which five different integrons were detected. In total different seven genes cassettes were found (*aadA1, aadA2, aadA5*) encoding enzymes that confers resistance to aminoglycosides and (*dfrA7, dfrA12*, *dfrA15, dfrA17*) conferring resistance to trimethoprim. These findings indicate that integrons of class 1 play an important role in resistance to trimethoprim and aminoglycosides in children less than five years.

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EVALUATION OF THE PATHOGENIC POTENTIAL OF *RICKETTSIA AMBLYOMMII* IN GUINEA PIGS (*CAVIA PORCELLUS*) AND PROTECTIVE IMMUNITY AGAINST *R. RICKETTSII*

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Rickettsia amblyommii belongs to the spotted fever group (SFG), which includes several vector-borne human and animal pathogens. Although *R. amblyommii* was determined non-pathogenic in one experimental animal model, there is serologic evidence of possible infection and mild disease in humans. Also, earlier experiments have shown protective immunity against R. rickettsii in guinea pigs that have been previously infected with SFG *Rickettsia*, but this has not been investigated with *R. amblyommii*. The aim of this study was to evaluate the pathogenic potential of *R. amblyommii* in guinea pigs and its capacity to generate protective immunity against *R. rickettsii*. Six guinea pigs were inoculated intraperitoneally with *R. amblyommii* and 2 controls with culture medium. Necropsies were performed in duplicate on infected animals at days 2 and 4 post-infection, and on infected and controls on day 13. Temperature and weight were evaluated and blood samples were drawn on days 0, 1, 2, 3, 4, 7, 9, 11, and 13. Blood and tissues were processed by PCR to detect the gltA gene, and end titers of anti-*R. amblyommii* IgG were determined by indirect immunofluorescence. To evaluate protective immunity, another 5 guinea pigs were infected with *R. amblyommii*; after 4 weeks, these 5 infected guinea pigs (IGP) and 3 control guinea pigs (CGP) that had not been infected previously were inoculated with pathogenic *R. rickettsii*. Titers of anti-*Rickettsia* IgG and clinical signs were evaluated. After infection with *R. amblyommii*, IgG titers reached 1:512 at day 13 post-infection. *Rickettsia amblyommii* was detected by PCR in testicles on day 2. Some guinea pigs showed orchitis without other signs of disease. In the protective immunity assay, anti-*Rickettsia* IgG end titers after *R. rickettsii* infection were lower in IGP than in CGP. *Rickettsia rickettsii* was detected by PCR in testicles of CGP only. IGP did not exhibit disease or had only transient fever, while CGP showed severe disease and two died. Results demonstrate that *R. amblyommii* from Costa Rica produced infection, antibody response, and signs of mild pathology in guinea pigs after an experimental infection. Although more studies are required, *R. amblyommii* showed pathogenic potential and should not yet be excluded as a possible cause of disease. Also, its capacity to generate protective immunity may modulate the epidemiology and severity of *R. rickettsii* infections in areas where both species coexist.

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ACE-DEX ENCAPSULATION OF ANTIGENS AND ADJUVANTS IS A POTENT DELIVERY PLATFORM FOR A *BURKHOLDERIA PSEUDOMALLEI* VACCINE

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Decades of modern research have failed to produce an effective vaccine against *Burkholderia pseudomallei*. There are several obstacles to a vaccine including lack of an appropriate target, lack of sufficient understanding of the immune response to *B. pseudomallei*, and lack of a vehicle capable of priming the appropriate arms of the immune system once the target is identified. Ace-DEX microparticles are a novel polymer carrier which is sensitive to acidic conditions; the microparticles are relatively stable at pH 7.4, but rapidly degrade after phagocytosis by antigen presenting cells. This acid sensitivity has been shown to yield enhanced CD8+ and CD4+ presentation of subunit antigen compared to other biomaterials. Ace-DEX encapsulation provides additional utility by allowing encapsulation of potentially toxic substances (if delivered freely) to be targeted to the site of action. Imidazoquinolines (e.g., resiquimod), for example are synthetic, FDA approved, immunostimulants that are agonists for TLR 7 and TLR 8, which are expressed within endosomal compartments of macrophages and multiple subsets of dendritic cells. Here we demonstrate that Ace-DEX encapsulation of antigens and adjuvants provide a potent delivery vehicle, producing a rapid and robust immune response inducing humoral and cell-mediated immunity. Mice were vaccinated with Ace-DEX particles encapsulating *B. pseudomallei* whole cell lysate and resiquimod on day 0 and 7 via sub-Q injection. On day 14 mice were challenged i.p. and followed for 26 days. Two groups had >90% survival up to day 13, and by the end of our study, two mice in each Ace-DEX-vaccinated group were sterile in all organs examined (blood, liver and spleen). All control mice succumbed to disease within 36 hours. Ace-DEX microparticles may represent a critical component to an effective *B. pseudomallei* vaccine.

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RESEARCH OF IMMUNOGENICITY OF EXPERIMENTAL SERIES OF POLYVALENT VACCINE AGAINST LEPTOSPIROSIS IN BOVINE USING LABORATORY ANIMALS

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The purpose of our work was to research immunogenicity of new polyvalent vaccine against cattle leptospirosis using laboratory animals - rabbits. For this research, we used an experimental inactivated and concentrated 6-valent vaccine against cattle leptospirosis produced in leptospirosis laboratory of the Institute for Veterinary Medicine, NAAS. Antibody titers' formation dynamics was studied in microagglutination test (MAG) in serum laboratory animals (rabbits) serum on the 25th day after vaccination. MAG was carried out using 6 strains of *Leptospira* included into the vaccine composition: Sejroe (serovars polonica and hardjo), Hebdomadis, Icterohaemorrhagiae, Grippotyphosa, and Tarassovi. Serum dilutions from 1:50 to 1:1600 were tested in duplicate. The dilution where > 50% of the *Leptospira* were agglutinated was considered as the antigen titer. "The vaccine was deemed active if the antibody titer to *Leptospira* was not less than 1:100 in the blood sera of at least four of five vaccinated rabbits, each weighing 3.0 - 3.5 kg. It has been established that specific *Leptospira* antibodies (Sejroe (hardjo) 1:320±40; Grippotyphosa 1:640±80; Tarassovi 1:1040±187; Sejroe (polonica) 1:1120±161; Hebdomadis 1:1280±161 and Icterohaemorrhagiae 1:800±0) formed in all test animals after 25 days since intramuscular injections of the vaccine dose of 0.75 ml. Titers of antibodies to all strains of *Leptospira* included into the vaccine composition in the blood serum of vaccinated rabbits significantly exceeded a critical value of 1:100. It is an evidence of high immunogenic activity of the vaccine, which was reached through the improvement of an existing manufacturing technology and application of advanced methods of standardization of leptospirosis antigens in the polyvalent vaccine composition.

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CHARACTERISTICS OF TRAVELERS TO ASIA REQUIRING COMPLEX MULTI-DOSE VACCINE SCHEDULES: RABIES AND JAPANESE ENCEPHALITIS PREVENTION

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Rabies and Japanese encephalitis (JE) pose risks to travelers to Asia, particularly those with longer and rural stays, outdoor activities, and visiting friends and relatives (VFR). Vaccines against both diseases require multiple doses and ≥21 days (rabies) or ≥28 days (JE) for completion. To describe interventions among travelers visiting affected areas in East (E), Southeast (SE), and South (S) Asia, we collected demographic and trip information for all patients seen during pre-travel consultations in BATMN clinics 03/08-07/10. Previous vaccination, travel reason, trip duration, and advice/immunizations were evaluated for travelers to E, SE, and S Asia. We calculated frequencies for categorical, and median and range for continuous variables. Of 15,317 patients, 5,091 (33%) traveled to E, SE, and S Asia. Of these 5,091, 52% were female and mean age was 36 years. Median trip duration was 17.8 days; 28% were traveling for >4 weeks. For rabies, only 5% had previously completed the series and 6% received complete series in the clinic. For JE, only 3% had previously completed the series, and 8% were immunized in clinic. Those traveling for education/research/missionary/ volunteer work had the highest proportion of rabies (17%) and JE (24%) vaccination; VFR travelers had the lowest (2%, 5%). Of 991 rabies vaccine-naïve travelers with trips >4 weeks, 583 (59%) were seen ≥21 days before travel. Similarly, of 994 JE vaccine-naïve travelers with trips >4 weeks, 461 (46%) were seen ≥28 days before travel. Most travelers received advice on vector precautions (97%); fewer were advised about rabies/animal contact (77-88%). In conclusion, frequency of rabies and JE vaccination was low among BATMN travelers, particularly for VFRs. Insufficient time to complete the series may have led to non-vaccination; travelers should schedule pre-travel consultations at least 4-6 weeks before travel. Cost may have also influenced vaccination decision. Health care providers should ensure rabies and animal bite prevention education in pre-travel consultations, in addition to emphasis on vector avoidance.

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DRUG REPURPOSING INITIATIVE FOR NEGLECTED TROPICAL DISEASES

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Repurposing of approved drugs for neglected tropical diseases (NTDs) has proven to be a critical strategy to serve unmet medical needs. Approved drugs are well characterized, do not require expensive development programs needed for new drugs and are frequently active against diseases not explored by their original drug developer/sponsor. The landscape of repurposed drugs for neglected tropical diseases will be reviewed, and promising new candidates will be discussed. FDA is developing a web-based program to both capture and share the experience of the medical community using already approved drugs repurposed to treat tropical diseases. Users will be able to submit and query individual cases or clinical studies on drugs repurposed to treat neglected tropical diseases. In addition to the published literature, this program can help consolidate global experience on drugs that are either effective or not effective against NTDs. Safety experience using repurposed drugs to treat NTDs will also be reported to this database. Drugs with promising performance may be adopted by sponsors for more formal drug development. This approach is likely to enrich the armamentarium of drugs for those neglected diseases which have little commercial appeal to traditional drug developers.

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SYMPTOMS AND CYTOKINE RESPONSES IN MALARIA MONO-INFECTION, DENGUE MONO-INFECTION AND MALARIA/DENGUE CO-INFECTION

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Over half of the world's population lives in an area endemic for malaria, dengue, or both. Despite overlapping endemicity, there is a lack of knowledge of the clinical presentation and host response associated with malaria/dengue co-infection. To identify symptoms and cytokine responses associated with co-infection, we conducted a retrospective matched comparative study within a febrile surveillance study conducted from 2002-2011 in the Peruvian Amazon Basin. For each co-infected subject (MAL-DEN), we selected 3 dengue mono-infected subjects (DENs) and 2 to 3 malaria mono-infected subjects (MALs) matched by location, time period (±3 months), gender, and dengue virus (DENV) serotype. Dengue was assessed using PCR and/or culture. Malaria was assessed using PCR and/or microscopy. We evaluated 20 symptoms using a standardized questionnaire. Seventeen inflammatory and regulatory serum cytokines and chemokines were quantified using Luminex xMAP technology. Matched comparison among MAL-DENs, MALs, and DENs was performed using conditional logistic regression for symptoms and random effects regressions for cytokine responses. Seventeen MAL-DENs (*Plasmodium vivax* in 14, *P. falciparum* in 3; DENV-1 in 2; DENV-3 in 15) were identified. Three symptoms were significantly different ($p<0.05$) when comparing the groups: 1) more abdominal pain in MALs vs DENs, 2) more myalgia in DENs vs MALs, and 3) more cough in MALs vs DENs. MALs had higher serum concentrations of interleukin (IL)-1β, IL-4, IL-6, IL-10, IL-12, IL-13, IL-17, granulocyte colony-stimulating factor, and γ-interferon ($p<0.05$) than either MAL-DENs or DENs. These differences remained after adjusting for age and days since symptom onset. In summary, MAL-DENs possessed a cytokine profile different from MALs but similar to DENs and did not have a higher frequency of symptoms compared to those with mono-infection. This suggests that many MAL-DENs may have had an acute DENV infection while also having asymptomatic chronic malaria, thereby creating a cytokine and symptom milieu similar to infection with DENV alone.

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HUMAN IMMUNE RESPONSES AFTER CONTROLLED EXPOSURES TO SAND FLIES

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An innovative approach to *Leishmania* vaccine antigen discovery is exploration of the sand fly vector salivary molecules. In animal models, an effective cellular immune response to uninfected vector saliva has resulted in amelioration/protection from clinical leishmaniasis after parasite challenge. We hypothesize that humans develop immune responses to vector saliva deposited in the skin with sand fly (SF) bites. Our objective was to evaluate the clinical and immunologic responses in healthy human volunteers after controlled exposures to uninfected *Phlebotomus duboscqi* (Pd), a vector of cutaneous leishmaniasis, and *Lutzomyia longipalpis* (Ll), a vector of visceral leishmaniasis. Subjects were healthy 18-50 year olds with normal serum IgE levels, seronegative to SF saliva, and without a travel history to endemic SF regions. Fifteen subjects received bites from colony-raised Ll, 14 from Pd, with up to nine sessions/year. A variety of bite site reactions were noted including wheal and flare, vesicles and papules, delayed large local reactions at 48 hours, chronic pruritic papules lasting>2 weeks, and reactivations of prior feeding sites with subsequent SF bite exposures. Four subjects were removed from the study for bite-related adverse events. Low levels of serum IgG antibodies to SF salivary sonicate developed in most subjects following multiple exposures. SF saliva stimulated PBMC produced IFNγ and IL10. Skin biopsies of SF feeding sites showed varied histology, most commonly mononuclear perivascular and perieccrine infiltrates with occasional eosinophils, correlating with the clinical severity of the skin reactions. SF bites resulted in both reactogenic and immunogenic responses with variability in both the clinical reactions to SF bites and the systemic immune response. Immediate and delayed allergic reactions occurred. We are currently pursuing *Leishmania* vaccine antigen discovery to identify and avoid the reactogenic components of SF saliva, while concentrating work on the immunogenic salivary molecules.

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AN ANALYSIS OF TRAVELERS TO ASIA SEEN IN THE BOSTON AREA TRAVEL MEDICINE NETWORK (BATMN)

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Asia is a popular travel region consisting of 5 sub-regions based on UN definition. We described travelers to Asia, compared them with travelers to other regions, evaluated differences in characteristics and interventions in the 5 sub-regions: South (S), Southeast (SE), East (E), Western (W) and Central (C) Asia. Demographic and trip information were collected for all travelers seen in BATMN clinics 03/08-07/10. We calculated frequencies and proportions for categorical variables and median and range for continuous variables. Travelers to Asia compromised 36% of travelers (5591/15307), with S Asia (35%) the most popular region and W and C Asia less visited (10%, 1%). The average age was 39y; travelers to W and C Asia were older (44y, 50y). Median trip duration was 17d (IQR 14-30); 28% planned trips >1mo and median time to departure was 23d (IQR 11-40). C Asia travelers had longer trips (26d; IQR 17-45); 49% traveled for >1mo and longer duration to departure (37d; IQR 15-52). Travelers were predominantly white (70%) or Asian (17%); a higher proportion to C Asia were white (90%). Travelers stayed in hotels (39%), hostels (31%) and local residences (28%). Tourism was the main reason for travel (46%), followed by VFR (18%) and business (18%). A higher percentage traveled for VFR (22%) and business (25%) to S Asia than other sub-regions. In C Asia, business (20%) and missionary/volunteer (14%) were also popular reasons for travel. Compared to travelers to other regions, Asia travelers were more likely to be male, older, born in Asia, more likely to travel for business and less likely for missionary/volunteer and have longer trip duration. Fewer received advice on swimming hazards, STIs and seat belts (83%, 68%, 87%) than other precaution topics. Azithromycin was prescribed more for self-treatment of diarrhea in travelers to S, SE and E Asia (79%, 65%, 48%) and ciprofloxacin to those visiting W and C Asia (63%, 77%). Atovaquone-proguanil was the most common anti-malarial prescribed for all regions. Typhoid immunization rates were higher in S Asia and SE Asia (77%, 75%), compared with E, W and C Asia (67%, 67%, 64%). JE and rabies vaccination rates were 6% and 8%. S Asia is the most popular of 5 Asian sub-regions to be visited by BATMN travelers. C and W Asia were less common destinations, their older age and longer trip duration may suggest different health risks such as road injuries, STIs. Pre-travel advice on swimming hazards, STIs, and seat belts need improvement.

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RECOMBINANT *LEPTOSPIRA*L IMMUNOGLOBULIN-LIKE A PROTEIN-BASED IGM ELISA ASSAY FOR THE DIAGNOSIS OF LEPTOSPIROSIS

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Diagnosis of leptospirosis performed by culture or microscopic agglutination test (MAT) is laborious and not practical in clinical settings. The objective of the current study was to evaluate the clinical implication of a new immunoglobulin M enzyme-linked immunosorbent assay (IgM ELISA) using recombinant leptospiral immunoglobulin-like A (LigA) protein in endemic areas. A prospective study was conducted in a national infectious disease hospital in Metro Manila, the Philippines. Plasma and urine samples were collected from patients with clinically diagnosed leptospirosis from November 2011 through October 2012; all samples were tested by MAT, loop mediated isothermal amplification (LAMP) for *Leptospira*l rrs, and LigA-based and Patoc-based IgM ELISAs. The case was defined based on laboratory findings; 1) specific antibodies were detected with a single titer of >=400 or at least a 4-fold increase in the MAT titer between paired samples, or 2) LAMP was positive at least in 1 plasma and/or urine sample. The ELISA optical density (OD) values were compared between laboratory confirmed cases and 100 blood donor samples, and receiver operating characteristic (ROC) analyses were performed. A total of 72 clinically diagnosed cases were enrolled in this study, and paired samples were available for 36 cases. The median duration from onset to the collection of the first sample (acute phase) and the second sample (convalescent phase) were 7 (range, 3 to 40) days and 11 (6 to 46) days, respectively. Among all cases, 39 were laboratory confirmed; 38 (97.4%) were male, and median age was 30 (19 to 64) years. 29 had water-contact history, and 1 died. The area under ROC curve of LigA-based and Patoc-based IgM-ELISA were 0.82 and 0.7, respectively ($p<0.05$). When the mean+3SD OD value of 0.23 was used as the cut-

off limit, the sensitivity and specificity of LigA-based IgM-ELISA were 92.9% and 88.3%, respectively in acute phase and 92.3% and 99%, respectively in convalescent phase. Among the laboratory confirmed cases, LigA-based IgM-ELISA OD value reached the peak at 11 days after the disease onset and then declined. In acute phase, presence of jaundice, absence of diarrhea, lower hemoglobin, and higher creatinine were significantly associated with LigA positive cases but not with LigA negative MAT positive cases. LigA based-IgM ELISA is a useful diagnostic tool for leptospirosis in clinical settings in endemic areas.

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EXPERIENCES WITH IMPLEMENTATION OF MALARIA RAPID DIAGNOSTIC TESTING AT PRIMARY HEALTH CARE LEVEL IN NIGERIA: IMPLICATIONS FOR SCALE-UP

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Nigeria commenced a phased programmatic deployment of rapid diagnostic tests (RDT) at the Primary Health Care (PHC) facility levels. However, against the backdrop of unexpected reactions to health intervention programmes like the polio vaccination campaigns, the acceptance, compliance and appropriate use of RDTs could not be assumed due to complex socio-cultural characteristics of the country. This implementation research was conducted to identify uptake, compliance and behavioural issues related to RDT use at the PHC level in Nigeria with their implications for scale-up of RDT deployment. A cross-sectional survey was conducted in 120 randomly selected PHCs across six states, across the six geopolitical zones of Nigeria in January 2013. Data on fever prevalence, malaria prevalence by RDT and adherence to test results were extracted from the medical records using a checklist covering Jun - Nov 2012. Compliance was the proportion of ACTs correctly prescribed in relation to RDT result. Health facility staff interviews were conducted to assess health workers (HW) prescription practices, knowledge and determinants of RDT use. A total of 1207 consecutive patient exit interviews to assess patient/caregiver's opinion of RDT use were conducted. There were 248,485 clinic attendees of which 129,272 (52.7%) presented with fever, but only 52,343 (39.8%) of these were tested using RDT kits with 31, 836 (60.8%) being positive for malaria. Of these30,674 (96.1%) received ACT while 8,418 (41%) of 20,507 RDT negative cases received ACT. Compliance rate was 81.7%. Of 118 HW's responses, about one-third each reported they would prescribe ACT (32.9%) or give antibiotics (29.8%) or refer (30.7%) when RDT is negative. Age of patients less than 5years (p=0.04) and "high" educational status of care givers (p=0.0006) were added determinants of health workers prescription of ACT to RDT negative patients. Overall, HWs had good knowledge of RDT. However in Enugu state, RDT was not being used because of a pervasive notion that it is not accurate. Among respondents who had RDT done, over 95% knew that RDT tested malaria, felt it was necessary and liked the test. The study demonstrated that RDT implementation at PHC level in Nigeria is feasible, safe, and acceptable with a significant compliance rate to RDT results. However pockets of challenges need to be addressed in the scale-up phase.

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ETIOLOGY OF ACUTE FEBRILE ILLNESS IN FOUR HOSPITALS IN HAITI, APRIL 2012 - JANUARY 2013

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Acute febrile illness (AFI) causes significant morbidity and mortality worldwide. In Haiti, little is known about the etiologies of AFIs; the national surveillance system relies primarily on syndromic diagnoses, and clinicians often treat AFI patients empirically. In April 2012, we established laboratory-based surveillance for AFI at four hospitals -- two in Port Au Prince, one in Artibonite and one in Southeast -- to determine the major etiologies of AFIs and to monitor seasonal trends of AFIs. We defined AFI as a documented temperature ≥37.5ºC in a hospitalized patient at or within 24 hours of admission. Trained nurses collected blood specimens from and administered questionnaires to up to 10 AFI patients a week at each hospital. At the hospital, specimens were tested by rapid diagnostic tests for dengue and malaria. Blood was centrifuged, and sera were sent to the national laboratory to be tested for leptospirosis by immunodot and typhoid fever by TUBEX TF. From April 3, 2012 through January 16, 2013, we enrolled 1,284 AFI patients. The median age was 24 (Range: 1 month-99 years), 441 (36.1%) were <5 years old, and 630 (49.2%) were male. The largest number of patients (393; 30.6 %) was from La Paix Hospital (HUP) in Port-au-Prince. Overall, 131/1015 (12.9%) samples tested positive by TUBEX TF; 32/1079 (2.9%) were positive for malaria (23 [71.8%] from HUP); and 34/885 (3.8%) were positive for dengue (18 [52.9%] from HUP). Only 15/862 (1.7%) AFI cases were positive for leptospirosis. The median age (range) of patients with typhoid, malaria, dengue and Leptospirosis was 27 (3 months-78 years); 16 (2 months-65 years); 26 (1 month-65 years); and 28 (6-78 years), respectively. There was no marked variability in seasonal activity among the four pathogens. In a 10-month period in four hospitals in Haiti, typhoid fever, malaria, dengue and leptospirosis all contributed to hospitalized AFIs, but collectively were associated with less than 20% of all AFI patients. Future surveillance should expand testing to investigate other causes of AFI.

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EFFECTS OF AN EDUCATIONAL INTERVENTION ON THE QUALITY OF THE INFORMED CONSENT OF PARTICIPANTS IN A CLINICAL TRIAL OF INTESTINAL HELMINTHS

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To obtain an informed consent is a crucial process for the ethical quality of clinical trials. However, participants of such researches generally have low comprehension regarding the information disclosed during the process of the informed consent. Also, they are under influences that interfere in their decision to participate in these researches, especially when such are carried out in developing countries. Therefore, it becomes necessary to create strategies to assure that the informed consent it will be a valid one, such as the accomplishment of educational interventions. Nevertheless, results from such interventions are controversial in affirming their impact in raising the informed consent quality, signalizing that further reflections, concerning different methods utilization, are needed. This study aim to analyze the impact of an educational intervention, based on a board game, on the quality of the informed consent from participants of an intestinal helminthiasis clinical trial that took part in the northeast region of Minas Gerais state, Brazil. The clinical trial goal was to evaluate the tolerability of adults residing in a helminthic endemic area to a functional

food with anti-helminthic properties. The study included 148 participants who were divided in two groups: experimental and control. On the first group, the subjects have joined on a board game before signing the informed consent form, while the second group just signed the document. In order to evaluate the informed consent quality, a structured questionnaire, that measures participant's knowledge concerning information of the informed consent and the presence of influences in the decision making process, was applied. Comparisons between the groups were done by Chi-Square and Mann-Whitnney test (significance level of 5%). Experimental group participants have showed larger knowledge regarding information of the informed consent and have presented lower influence over their decision to participate in the clinical trial. We have concluded that an educational intervention, that has applied a board game as an instrument, was able to improve the quality of the informed consent of participants in a clinical trial. It is also concluded that educational interventions should be associated to the process of informed consent achievement in order to favor a truthfully informed consent to participants of clinical trials.

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TRAVELERS' DIARRHEA RISK FACTORS AND INCIDENCE OF POST-INFECTIOUS IRRITABLE BOWEL SYNDROME (PI-IBS) IN A LARGE PROSPECTIVE COHORT OF DEPARTMENT OF DEFENSE BENEFICIARIES (TRAVMIL)

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There is limited prospective data regarding risk factors for and outcomes related to traveler's diarrhea (TD). Between 1/2010 and 11/2012, Department of Defense beneficiaries traveling outside the US for < 6.5 months were invited to participate in a prospective, multicenter cohort study - TravMil. Participants received a pre- and post- travel survey, and could opt into an illness diary completed during travel and a follow-up survey at 3, 6, 9 and 12 months post-travel. Standard definitions were used to assess for TD, and diagnosis of irritable bowel syndrome (IBS) was based on the modified Rome III criteria. Of the 838 participants who returned the questionnaires, 198 (24%) met criteria for TD: 9 (1.1%) had dysentery, 48 (5.7%) had acute febrile watery diarrhea and 141 (16.8%) had acute watery diarrhea. The highest incidence density rate (IDR) was found in Africa (7 cases per 100 person-weeks of travel (95% CI 5.4-9.4 cases per 100 person-weeks of travel)) and South Asia (6.1 cases per 100 person-weeks of travel (95% CI 3.6-9.8 cases per 100 person-weeks of travel)). Significant risk factors associated with TD in the multivariate model included: trip duration ≤ 2 weeks (IDR ratio 2.7 (95% CI: 1.9-3.8; p<0.01), leisure travel (IDR ratio 1.5 (95% CI: 1.1-2.1; p=0.02) and ingesting food prepared by street vendors or at home by local population (IDR ratio 1.4 (95% CI: 1.02-1.94; p=0.035). Among patients who did not have symptoms of IBS at baseline (n=803) and completed at least one follow-up survey (n=481), TD was not associated with an increased incidence of post infectious IBS at 12 months (IBS in patients with TD: 6.5% (7/108); IBS in patients without TD: 4% (15/373); relative risk 1.6 (95% CI: 0.67-3.85). Travelers' diarrhea remains a frequent occurrence among travelers to Africa and South Asia. The incidence of PI-IBS post-travel was similar to rates reported in prior studies. We were unable to demonstrate an association between TD and post-infectious IBS due to a small sample size.

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AN EPIDEMIOLOGICAL SURVEY OF PREGNANT WOMEN IN THE CHAGAS ENDEMIC REGION OF THE BOLIVIAN CHACO: A PRELIMINARY REPORT

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Chagas disease is a major health problem throughout Central and South America and is endemic across 60% of Bolivia. An estimated 26% of new cases are considered to be from congenital transmission. Current diagnostics and national screening protocols are estimated to miss 50% of congenital cases. Here we present the epidemiological data collected from a study of pregnant women admitted to Hospital Municipal Camiri in Camiri, Bolivia evaluating the prevalence of and risk factors for *Trypanosoma cruzi* infection. The women were recruited prior to giving birth and asked about their health history, socio-economic status, and risk factors for infection with *T. cruzi.* The mothers' venous blood was screened by the InBios Trypanosoma Detect Rapid Test. Mothers were considered to be *T. cruzi* infected if positive by Indirect Hemaglutination Assay and ELISA. To date, 174 women (mean age ±SD, 24.8 ±6.9) have entered the study of whom 79 have *T. cruzi* infection (45.4%). A significant association was shown between increasing age and *T. cruzi* infection in the mothers (p=0.0007). Overall, 37.6% of the women live in rural areas, and 27.9% reported living in a house infested with the triatomine vector. Women from rural areas were more likely to be infected with *T. cruzi* (p=0.038), and reported greater triatomine presence in their house (69.4% in rural vs 2.91% in urban areas). Women who lived more than 10 years in an infested house had a significantly higher rate of *T. cruzi* infection compared those who lived fewer than 10 years in an infested house (Odds ratio [OR] 4.45; 95% Confidence Interval [CI]: 2.21-9.02). Women who reported having a family member with Chagas or having a history of a triatomine bite were more likely to be *T. cruzi* infected (p=0.001, p<0.0001). Preliminary analyses suggest women of childbearing age living in rural areas near Camiri have a high prevalence of *T. cruzi* infection. Forthcoming data on congenital transmission rates in our unique and highly seropositive study group will allow multivariate analyses of risk factors for congenital transmission.

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THE POTENTIAL IMPACT OF IMPROVING APPROPRIATE TREATMENT FOR FEVER ON MALARIA AND NON-MALARIAL FEBRILE ILLNESS (NMFI) CASE MANAGEMENT IN UNDER-5S: A DECISION-TREE MODELLING APPROACH

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As international funding for malaria programmes plateaus, limited resources must be rationally managed. Although previously advocated, a policy of presumptive malaria treatment has led to widespread unnecessary prescription of antimalarials to non-malarial febrile illness (NMFI) cases. Hence it is critical to better understand how to implement interventions such as first-line Artemisinin Combination Therapies (ACTs) most effectively through an existing system, ensuring those who need treatment receive it, and that those who do not, are not needlessly treated. We developed a decision-tree tool to estimate the impact of improving health systems factors on rates of appropriate treatment for fever and on use of ACTs,

and to also evaluate the impact in Tanzania of the revised WHO malaria 2010 guidelines advocating diagnostic-led management. At baseline, 49% malaria cases attending a clinic receive ACTs (95% Uncertainty Interval: 40.6-59.2%) however 44% (95% UI: 35-54.8%) NMFI cases also receive ACTs. Increased treatment-seeking was the most effective step in increasing the proportion of all febrile cases correctly managed, but had no effect in improving case management of those patients attending the clinic. Provision of 100% ACT stock led to a 28.9% increase in malaria cases treated with ACT, but also increased overtreatment of NMFI, with 70% NMFI cases (95% UI: 56.4-79.2%) receiving ACTs and so an overall 13% reduction (95% UI:5-21.6%) in correct management of febrile cases. Increased availability or use of diagnostics had little effect on malaria management, but did significantly reduce NMFI overtreatment. Modelling the impact in Tanzania of the revised WHO guidelines, NMFI overtreatment decreased by 35% (95% UI: 31.2-39.8%), but malaria cases receiving ACTs reduced by19.5% (95% UI: 11-27.2%), due to a fourfold decrease in cases that were untested or tested false-negative (42.5% vs. 8.9%) and hence untreated. Multi-pronged intervention strategies were most effective to improve malaria treatment without increasing NMFI overtreatment. As malaria transmission declines, health system interventions must be guided by whether the management priority is an increase in malaria cases receiving ACTs (reducing the treatment gap), reducing ACT waste through unnecessary treatment of NMFI or expanding appropriate treatment of all febrile illness.

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ETIOLOGY OF INFECTIONS OF THE CENTRAL NERVOUS SYSTEM AMONG CHILDREN IN MBARARA, UGANDA

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Knowledge of etiological agents causing central nervous system (CNS) infections among children is essential to the development of guidelines for case management. This is especially true where there is little investigative capacity. We conducted a prospective descriptive study of the etiology of CNS infections in children two months to 12 years of age admitted to Mbarara Regional Referral Hospital with fever or a history of fever and at least one sign of CNS involvement. Clinical examination and biological sampling were performed upon admission. Pathogens were identified from CSF and blood samples following microbiological analysis, molecular diagnosis and serology. We diagnosed malaria using rapid diagnostic test and blood smears. Children were clinically assessed at discharge and then followed for any neurological sequellae.Between August 2009 and October 2012 we recruited 480 children with clinically suspected infection of the CNS, with a median age of 2 years (range 1month-5 years). The most important clinical symptoms at admission were prostration (78.5%), reduced consciousness (71.0%) and seizures (50.8%). Eighty-eight children (18%) died in the hospital. Malaria accounted for 166 (35%) of the laboratory confirmed diagnoses with a Case Fatality Ratio (CFR) of 13% while 49 (10%) of the children had culture or PCR-proven bacterial meningitis with a CFR of 16%. The most common pathogens were *Streptococcus pneumoniae* (33) followed by *Haemophilus influenzae* (7) and *Salmonella spp.* (6). Preliminary results of viral PCR and serology revealed 4 HHV6 and 1 mumps infections. No etiological agent could be identified in 198 patients (41%). Among those who survived, the proportions of neurological sequellae were 23% at discharge, 17% at one month and 11% at six months. Malaria is the main cause of CNS infections among children in Mbarara. These infections remain an important cause of mortality and morbidity and a treatment challenge due to diagnostic difficulties.

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INTRODUCTION OF ZINC WITH ORS FOR THE TREATMENT OF PEDIATRIC DIARRHEAS: THE GHANA CASE STUDY

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Key lessons learned through the public-private partnership in Ghana for increasing the use of ORS and zinc for the treatment of pediatric diarrheas will be described. Diarrheal disease is highly prevalent among children under five in Ghana, at a rate of about 20 percent, and accounts for an estimated 9 percent of childhood deaths. In 2011 the Government of Ghana adopted WHO/UNICEF guidelines recommending treatment of pediatric diarrhea with 10 days of zinc plus oral rehydration solution (ORS). The Ghana Health Service with USAID partners, USAID Strengthening Health Outcomes through the Private Sector (SHOPS) Project and Johns Hopkins Center for Communication Programs, used a collaborative approach to maximize availability of, demand for, and use of ORS plus zinc. Ingredients for success included: Public sector leadership to establish a conducive policy and regulatory environment; stakeholder engagement from both public and private sectors to develop training materials for health professionals and conduct joint training of trainers; training of private sector providers in diarrhea management including licensed chemical sellers, pharmacists and clinicians; grants to local pharmaceutical manufacturers to increase availability of quality, affordable pediatric zinc and marketing/detailing to private retailers and providers; a strategic and innovative mass media campaign increased awareness, demand and use; and grants to local NGOs to promote use of ORS plus zinc through interpersonal contact with caregivers and community mobilization activities. In 2012 mystery client survey and in-depth interviews, over 80% of providers mentioned zinc as their preferred diarrhea treatment and 65% actually prescribed zinc with ORS as treatment. Private sector sales of zinc tablets increased ten-fold after training providers and an additional 300% after the media campaign, treating over 1 million children with diarrhea during 2012. In a single year, significant progress in scaling up diarrhea treatment can be realized through targeted partnerships with the private sector.

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CLINICAL SIGNS AND SYMPTOMS ASSOCIATED WITH CHOLERA PATIENTS IN HAITI, 2012

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As of March 24, 2013, over 651,339 patients had been reported to the national cholera surveillance system in Haiti since the outbreak began in October, 2010. All patients with acute watery diarrhea who present to cholera treatment facilities (CTFs) are treated as presumptive cholera patients. We used data from a sentinel surveillance system for diarrhea in Haiti to identify signs and symptoms associated with cholera. We conducted surveillance for diarrheal illness in four hospitals in Haiti located in three departments: two in Ouest, one in Artibonite and one in Sud-Est. In each hospital, nurses recruited 10 patients per week who had at least 3 episodes of acute watery diarrhea within 24 hours with onset in the prior 7 days. Patients who had taken antibiotics were excluded. For each patient, we collected demographic and clinical information and a stool sample, which was sent to the National Public Health Laboratory where it was cultured for *Vibrio cholerae*. From April 1, 2012 -- December 12, 2012, we enrolled 921 patients, of whom 490 (53.2%) were male. The mean age was 23 years old (Range: 1 month–99 years), 187 (20.3%) were <5 years old, and 574 (62.3%) had cholera confirmed by culture. Compared to cholera-negative patients, cholera patients were more likely

to be severely dehydrated (69.1% vs. 41.0%; OR=3.2; 95% CI=2.3-4.4), to have had >10 episodes of diarrhea in a 24-hour period (57.1% vs. 32.2%%; OR = 2.8; 95% CI=2.0-3.8), to have vomiting (85.7% vs. 67.8%; OR =2.8; 95% CI=2.0-3.9), and muscle pains (31.4% vs. 16.5; OR=2.3; 95% CI=1.6-3.3). Both vomiting and 10 or more stools in a 24-hour period were more strongly associated with cholera among patients <5 years old than among older patients (OR 4.7 vs. 1.5 for vomiting; and OR 3.3 vs. 1.7 for ≥10 stools per day, respectively). In settings where diagnostic testing is not immediately available, evaluating certain signs and symptoms in patients with acute watery diarrhea may help clinicians more accurately identify patients with cholera.

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UNDERSTANDING DETERMINANTS OF INTERNATIONAL TRAVEL BEHAVIOR FOR PREVENTION OF IMPORTED DENGUE

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Travel medicine uses a risk assessment framework of social determinants of health for high-risk visiting friends and relatives (VFR) travelers to determine risk of travel-associated diseases. Such assessments are subject to bias and inaccuracy because these lack a social-ecological perspective of factors that influence travel behavior. Imported Dengue is a concern due to risk of secondary transmission and outbreaks in non-endemic regions. For U.S. travelers, Dengue prevention includes mosquito avoidance practices (MAP) and passive surveillance. Identifying determinants of MAP can improve Dengue prevention. A mixed-methods project identified and described determinants of MAP in U.S. VFR travelers. Next, a multi-case study of travel cohorts to Trinidad, Brazil and Thailand identified social/physical environmental influences on MAP in a cross-case content analysis. Finally, an interpretive phenomenology study described the meaning of going home for VFR travelers. Preceding pilot studies revealed determinants of intended MAP in a survey of travelers to Trinidad Carnival. Survey analyses included an exploratory factor analysis, Fisher's exact test, logistic regression and coding of open-ended questions. Factors associated with lack of intended MAP were carnival dedication (p=0.025) and feeling at home in the travel destination (p=0.007); VFR status was not significant (p=0.567). Semi-structured interviews revealed MAP are associated with risk perception, cultural familiarity and cultural embeddedness, irrespective of VFR status. Results included a *Cultural Embeddedness and MAP* model. Constructs include risk distractions, risk motivators, and objective and subjective decision-making for MAP. Multi-level prevention strategies would complement recommendations for MAP. Dengue prevention should include a Dengue Early Warning Surveillance System and fever-screening programs in high-risk cities and airports. Furthermore, VFR terminology does not accurately depict high-risk travelers, so adoption of the concept of '*Cultural Embeddedness*' should improve risk assessments of high-risk travelers.

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ENHANCING HEALTH CARE WORKER CAPABILITIES TO DETECT AND CARE FOR PATIENTS WITH MONKEYPOX

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Monkeypox has been a nationally notifiable disease in the Democratic Republic of Congo (DRC) since 2000, with oversight for surveillance and response activities provided by the *National Program for Monkeypox and Hemorrhagic Fevers*. In October 2010, the CDC Poxvirus Team initiated collaboration with the Ministry of Health and the Kinshasa School of Public Health to identify methods to enhance monkeypox surveillance and containment capacity, focusing on Tshuapa District in Equateur Province. In early February 2011 and again one year later, five representative health care workers from each of the 12 health zones in Tshuapa District attended a monkeypox surveillance training program. These individuals were expected to then replicate training for subordinates in their respective health care settings. Participants received training on monkeypox recognition, specimen collection, infection control, and in surveillance methods. In conjunction with the training program, an evaluation of training effectiveness and a brief needs assessment were performed using a self-administered written survey tool. Fifty-eight healthcare workers participated in the pre and post-knowledge evaluations. After training, more people correctly associated lymphadenopathy and deep well-circumscribed lesions (vs. superficial lesions) with monkeypox than prior (22/57 vs. 53/57, respectively). An improvement was also observed in the correct identification of vesicular fluid and lesion crusts (rather than blood) as preferred samples for laboratory testing. The lack of sampling kits, case forms, and personal protective equipment (PPE) were commonly indicated by survey respondents as impediments to MPX case investigations. When specifically asked how often the necessary PPE was available for collection of MPX samples, 47.7% responded 'sometimes' and 3.1% responded 'never', suggesting that lack of PPE is a notable barrier to the effective performance of monkeypox surveillance activities. This evaluation highlights several key areas for improvement of MPX surveillance in Tshuapa.

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SUSCEPTIBILITY TO, AND RISK FACTORS FOR, HEPATITIS E VIRUS INFECTION AMONG A REFUGEE POPULATION - SOUTH SUDAN, 2012

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Hepatitis E Virus (HEV) infection causes outbreaks in areas with poor water, sanitation and hygiene (WASH) conditions. Mortality rates range from 0.2% to 4.0%, but can reach 30% among pregnant women. In September 2012, an HEV outbreak was confirmed in Jamam refugee camp, South Sudan. At the time, there were approximately 15,000 refugees living in Jamam camp with 668 suspected/confirmed cases and

22 deaths. CDC was asked to assess the prevalence of infection and risk factors for HEV. A cross-sectional serosurvey was conducted in November 2012. Two individuals ≥3 years were randomly selected from a simple random sample of households. We collected serum for anti-HEV antibody testing and information on hygiene practices and exposures from each participant. We identified risk factors for HEV infection through a nested case-control analysis based on serology results; cases were IgM positive individuals while controls were unmatched individuals, negative for both IgM and IgG. A total of 443 individuals participated in the serosurvey; 21.7% had recent HEV infection (IgM positive), 24.1% had past infection (IgG positive), and 54.3% had no evidence of HEV infection (IgM and IgG negative). A total of 106 cases and 215 controls were included in the nested case-control study. Most cases were between 15-45 years and there was no significant difference between sexes. Individuals reporting close contact with a person with jaundice and those taking care of animals had 1.8 (95% CI: 1.1, 3.1) and 2.3 (95% CI: 1.03, 4.9) times the odds, respectively, of recent HEV infection compared to those unexposed. No other exposures were associated with infection. In conclusion, over 50% of Jamam refugees were susceptible to HEV infection four months after the outbreak started, highlighting the risk for continued transmission. Contact with a person with jaundice may play a role in HEV transmission in this population. The role of animal caretaking needs further investigation. Personal hygiene and WASH interventions should be scaled up in the face of a prolonged HEV outbreak.

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ECOLOGICAL AND EPIDEMIOLOGICAL STUDY OF VIRAL AND *RICKETTSIA*L PATHOGEN PREVALENCE IN TICKS AND MOSQUITOES IN THE NORTHERN PART OF AZERBAIJAN

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This project, which began in October 2012, aims to: conduct ecological and epidemiological studies of ticks, sandflies, and mosquitoes that may act as disease vectors of viral and rickettsial pathogens northern Azerbaijan; identify by PCR the viruses and rickettsia carried by these vectors; map vector and pathogen foci using Geographic Information Systems (GIS); and develop strategies for vector and disease surveillance and control based on these results. Data collected by this project will be used to test the hypothesis that understanding the ecology of arthropod disease vectors - ticks, sandflies, and mosquitoes -can assist public health authorities in anticipating the occurrence of the diseases they harbor and may transmit to humans and animals. Research will be conducted in northern regions of Azerbaijan, including Khachmaz, Guba, and Gusar, to investigate the following pathogens: Crimean-Congo hemorrhagic fever virus; tick-borne encephalitis virus; West Nile virus; Toscana virus; Sindbis virus; and various *Rickettsia* species, including that which causes Q-fever. Expected outcomes of this study: determination of the distribution of various tick, sandfly, and mosquito species within selected areas, including host and environment dependent distribution; deployment of up-to-date GIS technology to enhance surveillance, epidemiological, and analytical capabilities; full employment of DTRA-renovated facilities in Azerbaijan, including those located outside of Baku; exercise of knowledge gained from previous DTRA-sponsored research projects; application of modern molecular analysis techniques and expansion of project personnel's capabilities in these methods; and creation of an archival reference collection of arboviral and rickettsial genetic material for future use in identification, research, and training efforts. Initial sample collection was conducted in October 2012, with the quantification and identification of vectors collected still to be completed. We anticipate having preliminary testing results available by the date of the conference.

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EFFECTIVENESS OF LONG-LASTING PERMETHRIN IMPREGNATED UNIFORMS FOR TICK BITE PREVENTION AMONG FORESTRY, PARKS AND WILDLIFE WORKERS IN NORTH CAROLINA

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Outdoor workers, because of frequent exposure in tick habitats, are at high-risk for tick-borne diseases. Adherence to NIOSH recommended tick-bite prevention methods is poor. While self-applied permethrin treatment of clothing is highly protective against many arthropod vectors, the need for frequent reapplication lessens adherence. We evaluated the protective effectiveness of long-lasting permethrin impregnated (LLPI) uniforms among a cohort of North Carolina outdoor workers. A double-blind randomized controlled trial was conducted to determine the effectiveness of LLPI uniforms for the prevention of tick bites among outdoor workers from North Carolina State Divisions of Forestry, Parks and Recreation, and Wildlife. 159 subjects were randomized; uniforms of participants in the treatment group were factory-impregnated with long-lasting permethrin while control group uniforms received a sham treatment. Participants continued to engage in their usual tick-bite prevention activities and provided weekly tick bite logs during two tick seasons. Questionnaires were completed annually. Study subjects reported 1,045 work-related tick bites over 5,251 person-weeks of follow-up. The mean number of reported tick bites in the year prior to enrollment was similar for both the treatment and control groups, but markedly different during the study period. The effectiveness of LLPI uniforms for the prevention of work-related tick bites was 82.8% (95% Confidence Interval (CI): 67.7% to 90.8%) for the first year of follow-up and 38.4% (95%CI: -51.4% to 74.0%) for the second year of follow-up. These results indicate that LLPI uniforms are highly effective for at least one year in deterring tick bites in the context of existing tick bite prevention measure usage by outdoor workers.

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PREVALENCE OF *RICKETTSIA, EHRLICHIA* AND *BORRELIA* IN ARTHROPODS IN GEORGIA

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Rickettsia, Borrelia and *Ehrlichia* are arthropod-borne pathogens widely distributed throughout the world. These pathogens cause diseases with similar clinical signs, they can share the same transmitting vector; co-infection is also possible that makes difficult to diagnose. Preliminary study showed a relatively high infection rate for spotted fever group *Rickettsiae* among ticks in Georgia. No studies had been conducted on *Borrelia* and *Ehrlichia* in vectors. The goal of the study was to evaluate the prevalence of *Rickettsia, Ehrlichia*, and *Borrelia* species in Georgia. 500 single species vector pools, representing 13 species collected from 3 regions of Georgia were studied using qPCR. Analysis of the results revealed that 47% of sample pools were positive on *Rickettsia*, 23% on *B. burgdorferi*; 19% of the pools were positive for both *Rickettsia spp.*, and *B. burgdoferi*. Distribution of *Rickettsia* was similar in all regions. The distribution of *B. burgdorferi* was not uniform. *Rickettsia* and *B. burgdorferi* were found in all tick species with the exception of ticks of the genus *Argasidae* (possibly due to small number of these vectors - 32 tick samples were tested). Of all tick species studied, minimum infection rates (MIRs) for both *Rickettsia* and *B. burgdorferi* were highest for *Hyalomma* genus. For *Rickettsia, H.*

plumbeum had the highest MIR (2.19%); the highest MIR (2.15%) for *B. burgdorferi* was observed in H. aegyptum. The flea pools that were inadvertently analyzed in this study were *Rickettsia* and *B. burgdorferi* negative, but this does not discount the possibility that fleas in Georgia carry these pathogens as the sample size for these vectors was extremely limited. This study is still ongoing and more samples from other regions of Georgia are under investigation. Screening ticks for these bacterial agents has provided insights regarding the distributions and endemicity of potentially pathogenic and emerging tick-borne agents. Continued study and monitoring will play an important role in public health assessment for related disease risks.

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IDENTIFYING A REFERENCE GENE FOR *RICKETTSIAE* USING *RICKETTSIA BELLII* AS A MODEL

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Rickettsioses are caused by obligate intracellular bacteria transmitted by hematophagous arthropods such as ticks. In rare occasions, ticks have been documented to be superinfected with two spotted fever group and an ancestral group rickettsia, as reported previously. Tick-borne rickettsioses are an emerging global health concern. *Rickettsia spp.* formerly recognized as nonpathogenic maybe contributors to the emergence of rickettsioses. With the emergence of tick-borne rickettsioses, there is a need to understand *rickettsiae* and how they respond to their arthropod and human host and to the environment at a molecular, cellular, and immunological level. In this research, we focus on using *Rickettsia bellii* RML-369C, a non-pathogenic bacterium, as a model to understand the transcriptional control as it grows in tick and mammalian cell culture. *R. bellii* is safer to handle and grow in laboratory settings, and grows voraciously in ticks and mammalian cell culture similar to pathogenic *rickettsiae*. Our overall aim is to study the transcriptional response of *rickettsiae* when they first infect a tick or mammalian host in nature to understand how they respond during infection and establishment of their hosts. This research aims to identify stable reference genes to analyze transcriptional responses of *R. bellii* grown in ticks and mammalian cells as a laboratory replica of rickettsial infection in nature. We used a two-step quantitative reverse transcription PCR to screen 5 housekeeping genes: *atpB*, *gltA*, *gyrA*, *infB*, and *tlc5* for use as reference genes. Out of the 5 genes assessed using Normfinder, we identified *gyrA* as the most stablely expressed gene throughout a 72 hour post infection growth period in tick cell culture. A best combination of *gyrA* and *tlc5* was identified as being suitable for transcriptional analysis.

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VIRAL AND *RICKETTSIA*L SCREENING OF IMMATURE TICKS COLLECTED IN MISSOURI

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The novel phlebovirus, *Heartland virus*, was identified in two patients from Missouri who had both been bitten by ticks 5 to 7 days before the onset of their symptoms. In September 2012 our laboratory group went on a collection trip to Missouri with the goal of isolating *Heartland virus* in ticks. We identified three geographically relevant collection sites. At the three locations 1267 larval ticks and 2 nymphal ticks were collected. The ticks were identified by morphology and then homogenized for pathogen screening. Of the collected ticks there were 93.9% *Dermacentor albipictus*, 5.8% *Amblyomma americanum*, and 0.3% *Ixodes scapularis*. Vero, VeroE6, and DH82 cells were infected with the homogenized ticks however no CPE was detected. Primers specific for *Heartland virus*, *Powassan virus*, and *Deer tick virus* were used in the viral PCR screening, but none of the samples from the collection sites were positive for virus. DNA extracts were screened for bacterial pathogens, and at one location a larval and a nymphal pool of ticks were both *Rickettsia*-positive. Sequence analysis demonstrated that there was 100% sequence identity between the two positive samples and the *Candidatus Rickettsia amblyommii ompA* and *citrate synthase* genes. Morphologic tick identifications were verified by mitochondrial 16S rRNA sequence analysis which confirmed that the *R. amblyommii*-positive samples were isolated from *A. americanum* ticks. Our data indicates the occurrence of transovarial transmission of *R. amblyommii*, as evident by positive larval ticks collected at a single location. To date no definitive role has been defined for *R. amblyommii* in human pathogenesis, but a recent study has shown that *A. americanum* ticks parasitizing humans are frequently infected with *R. amblyommii* (Jiang et al., 2010). Because other *Rickettsia* species, such as *R. parkeri*, were initially thought to be endosymbionts but were later shown to be pathogenic, it is important to continue evaluating the potential public health threat that *R. amblyommii*-infected *A. americanum* ticks pose to the humans they parasitize.

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DETECTION OF A *RICKETTSIA* CLOSELY RELATED TO *R. MONACENSIS* IN *IXODES BOLIVIENSIS* FROM COSTA RICA

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Rickettsia monacensis was officially described in 2002 from *Ixodes ricinus* ticks in Germany. It has now been detected in most areas of Europe and has been associated with Mediterranean spotted fever-like human rickettsiosis in Spain and Italy. *I. pacificus* and *I. scapularis* from North America also contain similar *Rickettsia sp.*, which have not been fully described. We report the presence of a *Rickettsia sp.* in *I. boliviensis* from Costa Rica that is very closely related to *R. monacensis*. In February 2012, ticks were collected from domestic dogs in the province of Heredia, Costa Rica. Specimens of the same species and from the same dog were pooled, and DNA was extracted. Pools were analyzed by *Rickettsia spp.* specific PCRs that detect fragments of the gltA, htrA, ompA, and ompB genes. Amplicons from positive pools were sequenced, and BLAST searches and phylogenetic analyses were performed. *I. boliviensis* were collected from 6 of 10 dogs evaluated. DNA of *Rickettsia spp.* was detected in all 6 pools of *I. boliviensis*. At least 2 of the tick pools contained gltA fragments that were 100% homologous with each other, and BLAST analyses determined 99.7% (365/366) homology to *R. monacensis* IrR/Munich. BLAST and phylogenetic analyses of gltA, ompA, and htrA amplicons confirmed that the *Rickettsia sp.* IbR/CRC present in *I. boliviensis* ticks from Costa Rica groups closely with *R. monacensis* and *Rickettsia sp.* from *I. pacificus* and *I. scapularis*. This is the first detection of a *Rickettsia* in *I. boliviensis* ticks of Central America. Further analyses are required to determine if *Rickettsia sp.* IbR/CRC is a genotype of *R. monacensis*, a genotype of the *Rickettsia sp.* in *Ixodes* from North America, or a different species yet to be described. Considering that *I. boliviensis* can bite humans and that *R. monacensis* and other closely related species have been associated with human disease, it is important to characterize and determine the pathogenic potential of this *Rickettsia*.

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MODELING DIFFERENTIAL INVASION TRAJECTORIES OF TICK-BORNE DISEASES ACROSS THE NORTHEASTERN U.S.

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The ongoing invasion of two tick-borne diseases, Lyme disease and human babesiosis, in the Northeastern United States presents a significant public health risk as well as a unique opportunity to study the dynamics of ongoing pathogen emergence. Although both pathogens share the

same tick vector and an overlapping community of vertebrate reservoir hosts, expansion of babesiosis has markedly lagged that of Lyme and is endemic in a much smaller range. Invasion of tick-borne pathogens is a product of local, small-mammal mediated spread and long-distance, bird-mediated spread. However, the relative contribution of different hosts to pathogen dispersal and the factors driving the differential expansion trajectories for the two pathogens remain unknown. We use a hierarchical Bayesian framework for testing mechanistic hypotheses that describe the spatio-temporal distribution of human cases of each disease across the Northeast. The model is parameterized with data compiled from state and county health departments from 1984-2011 for Lyme disease and from 1990-2011 for babesiosis. Three model structures are developed for comparison. Specifically, we examine the relative importance of local versus long-distance spread processes for predicting invasion dynamics of the two diseases. We also identify climate, landscape and vector biology factors significant in predicting pathogen diffusion. The best-fitting model structure included both spatially variable local diffusion and long-distance spread. Diffusion of babesiosis was found to be consistently slower than that of Lyme across the Northeastern emergence focus. This data-driven mechanistic framework is a simple but accurate approach to studying the invasion dynamics of pathogens maintained in complex ecological cycles such as the tick-borne infections presented here. Our method can be used to predict sites of probable invasion, thus identifying spatial targets for enhanced surveillance and control measures.

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PHLEBOTOMUS ORIENTALIS SALIVARY ANTIGENS - IDENTIFICATION, CHARACTERIZATION AND EXPRESSION

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Sand flies (*Diptera: Phlebotominae*) are vectors of *Leishmania* (Trypanosomatidae), the causative agents of cutaneous and visceral leishmaniasis. During the blood feeding, sand fly females inject saliva into the host skin to overcome host haemostatic mechanism. Repeated exposures to sand fly saliva elicit anti-saliva antibodies that could be used in epidemiological studies as a marker of exposure to assess the risk of *Leishmania* transmission and the effectiveness of anti-vector campaigns. The anti-saliva immunity has been also shown to protect the host from *Leishmania* infection, thus salivary proteins are considered as candidates for transmission blocking vaccine. The main aim of this study was to characterize and express salivary gland antigens of *Phlebotomus orientalis*, the important vector of life-threatening visceral leishmaniasis in East Africa. The major antigens were determined by SDS-PAGE and immunoblot using antibodies from dogs and humans repeatedly bitten by this sand fly species.. Based on the cDNA library and mass spektrometry (MALDI TOF-TOF), eight antigens from five different protein families were identified. All of these proteins with molecular weight ranging from 26 kDa to 42 kDa are antigenic for dogs but only four (apyrase, yellow-related protein, antigen 5-related protein, D7-related protein) are antigenic for humans. These four antigens were expressed in the bacterial expression system. Recombinant products will be compared in their ability to bind specific antibodies in sera from hosts repeatedly bitten by *P. orientalis* to select candidate antigen(s) usefull for larger epidemiological studies.

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EFFECTS OF FOREST PATCH SIZE ON ABUNDANCE OF DEER TICKS (*IXODES SCAPULARIS*) AND RECOGNITION OF RED-BACKED VOLES AS MAJOR RESERVOIRS OF *BORRELIA* AND *ANAPLASMA* IN GRAND FORKS COUNTY, NORTH DAKOTA USA

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Geographic range of the deer tick, *Ixodes scapularis*, has expanded in recent years. Deer ticks are forest ticks and historically considered not to occur as far west as North Dakota. However, a statewide survey conducted in 2010 found breeding populations of deer ticks in the Northeast region of the state. This region is under intense agricultural production but within the expanse of field crops there are islands of forested areas. In 2012 six forested areas in Grand Forks County, ranging in size from 7 to 349 hectares, were surveyed for deer ticks. Ticks were collected via dragging and from small mammals. There was a significant positive correlation between forested patch size and 1) abundance of questing adults and 2) intensity of larval ticks on infested hosts. Of 1,036 deer ticks collected, the vast majority (98%) were found only at the two largest sites. However, there was no significant difference among sites in the total abundance of small mammals (mean=0.25 per trap-night), suggesting that all the forested patches regardless of their size, had sufficiently abundant hosts to sustain tick populations. The small mammal fauna at the largest forest patch (n=69) was comprised mostly of *Peromyscus* (38%) and red-back voles, *Myodes gapperi* (58%). Although the prevalence of ectoparasitism for *Peromyscus* mice (88%) was significantly greater than for *M. gapperi* voles (60%), when engorged larval ticks were detached from the rodents and assayed for pathogens (i.e., xenodiagnoses), there were no significant differences in xenopositivity between infested *Peromyscus* mice and *M. gapperi* voles for either *Borrelia burgdorferi* (overall 6% xeno-positive animals) *or Anaplasma phagocytophilum* (overall 6% xeno-positive animals). Deer ticks have become established in discreet foci within northeastern North Dakota and the role of *M. gapperi* voles as reservoirs of Lyme disease and anaplasmosis in this region warrants closer scrutiny.

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ENTEROBIUS VERMICULARIS: A FIVE YEAR EXPERIENCE FROM AN INNER CITY HOSPITAL IN THE BRONX

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Enterobius vermicularis (Pinworm or oxyuriasis) is an intestinal nematode infection commonly reported in school-age children in the United States. Reports of pinworm in the immigrant populations are emerging in the literature. Jacobi Medical Center, located in the Bronx, serves a large immigrant population. Clinical presentation, treatment and country of origin are described in patients with *E. vermicularis* infections presenting over a 5 year period to our institution. Twenty two patients were identified. Ten males (46%) were seen with mean age 17 (SD$\pm$ 17). Of these patients 20 (91%) were immigrants and of these, 6 (30%) patients gave a history of recent travel history to visit friends and family. The majority of patients seen were from Albania (9, 41%), other countries included Yemen (4, 18.2%), Morocco (4, 18.2%), Mexico (1, 5%), Pakistan (1, 5%) and Ecuador (1, 5%). Twenty patients (91%) were symptomatic. Pruritis ani was reported in 11 (50%) patients. Work-up was initiated for nonspecific abdominal pain in 8 (36%) patients and 4 (18%) patients were evaluated for enuresis. The scotch- tape was positive in 20 patients (91%). Of the 18 stools examined for ova and parasites, 4 (22%) revealed pinworm. Eighteen (82%)patients had other intestinal parasites detected on stool exam. *Dientamoeba fragilis* was found in 8 (36%) patients. The mean absolute eosinophil count was 0.5 (SD$\pm$ 0.5). Six (27%) patients had past history of pinworms which may represent recurrent infections, athough treatment failure cannot be excluded. Ten (50%) had a family members infected. Albendazole was administered to